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On the crystal structure of $TiOSO_4H_2O$

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With 6 figures in the text

Tetravalent titanium ions are known to hydrolyze easily in water solutions, often yielding insoluble products of basic salts or hydrated oxides. However, in the chemical literature, the data on the hydrolysis of Ti^{4+} are very meagre and no crystal structure determination of a basic titanium compound seems to have been published, though many investigations on titanates have appeared. It thus seemed interesting to determine the structure of such a compound and we chose the titanium oxide sulphates as a suitable object for the investigations.

A number of titanium(IV) oxide sulphates are described in *Gmelin's Handbuch* (1). Among them there is one, $TiOSO_4(H_2O)_2$, which is said to be crystalline and attempts were made to prepare it according to the methods quoted. They were not successful since they only gave needle-like crystals too small to be investigated by X-ray single crystal methods. However, during the preparation of basic thorium, uranium, and cerium compounds (2–7), hydrothermal hydrolysis of solutions has proved to be very convenient for synthethizing crystals of sufficient size. By using such methods also on titanium(IV) sulphate solutions, it was possible to prepare crystals big enough to give Weissenberg photographs. The formula of the compound obtained was found to be $TiOSO_4H_2O$. It was prepared earlier by Kirkham and Spence, who describe it as a fine-grained powder (1). They made it by a method rather similar to that described below, though the temperature for the reaction was lower.

Preparation of $TiOSO_4H_2O$

$TiOSO_4(H_2O)_2$ was prepared according to Pamfilov and Khudyakova (8). The small needle-like crystals obtained were mixed with sulphuric acid (60 per cent by weight), sealed in a thick-walled Pyrex tube and heated to 160°C for three weeks. The tube was then rapidly cooled and opened. The precipitate of $TiOSO_4H_2O$ was filtered off, washed with water several times and dried in air.

The crystals are small colourless rods which are very slightly soluble in water or dilute acids. In concentrated acids they dissolve, but very slowly.

The largest crystals obtained were about 0.08 mm in length and 0.02 mm in diameter. Crystals of that size were, however, only prepared once—several repeated syntheses with the same method were not successful and only gave crystals that were considerably smaller.

Analysis

A sample dried at 100°C for two hours without any loss of weight was decomposed by fusion with sodium and potassium carbonates. After cooling, the melt was leached with water and dilute nitric acid. The residue was filtered off. The combined filtrates were then made slightly alkaline with dilute ammonia and the small precipitate formed (TiO_2aq) was filtered off. The solution was again acidified with nitric acid and treated with a barium nitrate solution. The precipitate of $BaSO_4$ was filtered off, washed, ignited, and weighed. From its weight the amount of *sulphur trioxide* in the crystals was calculated.

The amount of *titanium dioxide* in the crystals was determined by igniting a weighed sample in a platinum crucible at 1000°C for an hour and weighing the residue.

The *water* was determined by a modification of Brush and Penfield's method as described by Hartwig-Bendig (9–10).

The *density* of the crystals was determined from their loss of weight in benzene.

	Found	Calculated for $TiOSO_4H_2O$
% TiO_2	44.6	44.89
% SO_3	45.2	44.99
% H_2O	10.3	10.12
Density	2.71	2.744 (cf. below)

X-ray diffraction data

From rotation (around [100], [010], and [001]) and Weissenberg photographs (0*kl*, 1*kl*, 2*kl*, *h*0*l*, *h*1*l*, *h*2*l*, *hk*0, *hk*1, *hk*2) taken with Cu *K* radiation it was concluded that $TiOSO_4H_2O$ is orthorhombic with $a = 9.7$ Å, $b = 5.1$ Å, and $c = 8.6$ Å (the *b*-axis coincides with the needle axis of the crystal). More accurate values for the cell dimensions were obtained from powder photographs in focusing cameras of Phragmén type, cf. Table 1 (Cr *K* radiation. $\lambda_{\mathrm{Cr}\,K\alpha_1} = 2.28962$ Å):

$$a = 9.788 \pm 0.005 \text{ Å}$$
$$b = 5.120 \pm 0.003 \text{ Å}$$
$$c = 8.598 \pm 0.005 \text{ Å}$$
$$V = 430.9 \text{ Å}^3$$

From the volume of the unit cell and the observed density of the crystals we conclude that there are $3.9_5 \approx 4$ formula units in the unit cell.

The reflections in the Weissenberg photographs were recorded photographically using the multiple film technique. Their intensities were estimated visually but the accuracy in the estimations was rather low since the photographs were poor due to the smallness of the crystals prepared. The following reflections were found to be systematically absent:

$h00$ with h = odd
$0k0$ with k = odd
$00l$ with l = odd

Table 1. Powder photographs of $TiOSO_4H_2O$. Phragmén cameras. CrK radiation.

$h\,k\,l$	$10^4 \sin^2\theta$ calc	$10^4 \sin^2\theta$ obs	I calc	I obs
1 0 2	847	834	320	w
2 1 0	1048	1038	780	m
0 1 2	1210	1215	0	w –
2 1 1	1226		137	
2 0 2	1258	1250	1	vvw
1 1 2	1347	1338	194	w
3 0 1	1410	–	0	–
3 1 0	1733	1724	0	m
1 0 3	1734		313	
2 1 2	1758	–	20	–
3 1 1	1911	1899	44	vw
3 0 2	1942	–	0	–
0 2 0	2002	1990	177	vw
0 1 3	2098	–	0	–
1 2 0	2139	–	0	–
2 0 3	2145	–	2	–
0 2 1	2180	2171	0	vvw
4 0 0	2191	–	1	–
1 1 3	2235	2228	211	w
1 2 1	2316	–	1	–
4 0 1	2369	2358	88	w
3 1 2	2443	2433	90	vw
2 2 0	2550	–	0	–
2 1 3	2646	2640	4	vvw
4 1 0	2692	2707	7	vvw
0 2 2	2712		34	
2 2 1	2727		6	
3 0 3	2830	2836	29	vvw
0 0 4	2840		0	
1 2 2	2849		3	
4 1 1	2869	2872	48	vw
4 0 2	2901	(2918)	11	(vvw)
1 0 4	2977	2982	80	w
3 2 0	3235	–	0	–
2 2 2	3260	–	0	–
3 1 3	3330	3325	176	w
0 1 4	3340		0	
2 0 4	3387	3400	120	w
4 1 2	3402		0	
3 2 1	3412		152	
1 1 4	3477	(3480)	20	(vw)
0 2 3	3596	3596	0	vw
5 0 1	3599		42	
1 2 3	3736	3735	116	w
4 0 3	3789	–	3	–
2 1 4	3888	3900	10	vvw
5 1 0	3924	–	0	–
3 2 2	3945	3948	212	vw
3 0 4	4072	–	0	–
5 1 1	4102	(4095)	44	(vw)
5 0 2	4134	(4124)	0	(vvw)
2 2 3	4147		14	
4 2 0	4193	4194	252	w
4 1 3	4289	4288	48	w
4 2 1	4371	4357	79	vw
3 1 4	4573	(4580)	17	(vw)
1 0 5	4574		16	
5 1 2	4634	(4640)	0	(vvw)
1 3 0	4642		0	
0 3 1	4682	–	18	–
1 3 1	4819	4824	36	w
3 2 3	4832		194	
0 2 4	4842		0	
4 2 2	4903	4899	292	vw
6 0 0	4930	–	1	–
0 1 5	4938	4939	389	w +
1 2 4	4979	4984	0	w +
2 0 5	4985		246	
5 0 3	5021	–	0	–
4 0 4	5031	5044	45	vw
2 3 0	5052		275	
1 1 5	5074	–	8	–
6 0 1	5108	5094	58	vw
0 3 2	5215	–	0	–
2 3 1	5230	–	40	–
1 3 2	5351	5353	79	vvw
2 2 4	5390	–	5	–
5 2 0	5426	–	0	–
6 1 0	5431	–	0	–
2 1 5	5485	–	8	–
5 1 3	5515	5522	78	w
4 1 4	5525		53	
5 2 1	5597	5605	122	w
6 1 1	5602		36	
6 0 2	5634	–	2	–
3 0 5	5663	–	1	–
3 3 0	5731	–	0	–
2 3 2	5756	–	14	–
4 2 3	5784	(5797)	14	(vw)
3 3 1	5908	–	20	–
3 2 4	6067	6077	79	vw
0 3 3	6095		3	
5 2 2	6129	6129	144	w
6 1 2	6134		194	
3 1 5	6163	(6152)	1	(vw)
1 3 3	6232	6226	109	vvw
5 0 4	6256	–	2	–
0 0 6	6382	–	18	–
0 2 5	6432	–	0	–
3 3 2	6440	6449	40	vvw
1 0 6	6519	6524	1	vvw
6 0 3	6520		65	
1 2 5	6569	–	4	–
4 0 5	6621	–	4	–
2 3 3	6642	–	5	–
4 3 0	6688	–	5	–
5 1 4	6756	6758	68	vw

Table 1 (*continued*)

h k l	$10^4 \sin^2\theta$ calc	$10^4 \sin^2\theta$ obs	*I* calc	*I* obs	*h k l*	$10^4 \sin^2\theta$ calc	$10^4 \sin^2\theta$ obs	*I* calc	*I* obs
4 3 1	6866		12		7 1 1	7380	7377	144	w
7 0 1	6880	6880	69	vvw	4 3 2	7397	–	0	–
0 1 6	6882		0		7 0 2	7412	–	1	–
6 2 0	6925	–	7	–	2 1 6	7429	7435	48	vvw
2 0 6	6929	–	1	–	1 3 4	7473	–	10	–
2 2 5	6979	6977	374	w	3 0 6	7613	–	18	–
5 2 3	7015	–	32	–	6 2 2	7634	–	0	–
1 1 6	7019	–	8	–	3 2 5	7663	–	20	–
6 1 3	7020	–	3	–	6 0 4	7761	–	0	–
4 2 4	7025	(6999)	96	(vw)	5 0 5	7852	–	2	–
6 2 1	7102	7119	44	vw	2 3 4	7883	–	4	–
4 1 5	7121		78		7 1 2	7912	7904	12	vvw
7 1 0	7203	–	0	–	5 3 0	7920	–	0	–
3 3 3	7327	7319	73	vvw	0 4 0	7999	8000	240	vvw
0 3 4	7336	–	0	–					

The horizontal lines mark the boundaries between the angular ranges of the three focusing cameras used.

The values of $\sin^2\theta_{calc}$ have been calculated with $\lambda_{Cr\,K\alpha} = 2.2909$ Å for the low and medium angle cameras and with $\lambda_{Cr\,K\alpha_1} = 2.28962$ Å for the high angle camera.

Reflections systematically absent in space group no. 19 $P2_12_12_1$ have been omitted. Reflections coinciding with β-reflections are given in brackets.

The intensities were calculated from the expression $I_{calc} = 0.1\,(p/8)\,|F|^2$, where p is the number of equivalent reflections and F the structure factor. In the photographs there seems to be an orientation effect, favouring reflections with low k.

This is characteristic of space group no. 19 $P2_12_12_1$ (11). However, reflections $hk0$ with h = odd and $0kl$ with $k + l$ = odd are very weak, showing that the lattice has a pseudo-symmetry according to the space groups no. 62 *Pnma* or no. 33 $Pn2_1a$ (11). The weak reflections are also visible in powder photographs taken in a Guinier focusing camera with monochromatic Cu $K\alpha$ radiation.

Throughout the determination of the atomic parameters, the real space group ($P2_12_12_1$) was considered but the idealized structure arrived at finally fitted in with the more symmetric space group *Pnma*.

Positions of the titanium and sulphur atoms

According to space group no. 19 $P2_12_12_1$ all the atoms in $TiOSO_4H_2O$ should be arranged in fourfold positions 4(a): x, y, z; $\frac{1}{2} - x, \bar{y}, \frac{1}{2} + z$; $\frac{1}{2} + x, \frac{1}{2} - y, \bar{z}$; $\bar{x}, \frac{1}{2} + y, \frac{1}{2} - z$.

To determine the parameters of the titanium and sulphur atoms, the Patterson projections $P(upw)$ and $P(pvw)$ were calculated using the visually estimated intensities, corrected according to Lu (12). The results are given in Fig 1. As is seen, $P(pvw)$ shows large maxima only for $v = 0$ or $v = \frac{1}{2}$. This implies that the titanium and sulphur atoms should be situated in, or close to, planes $b/2$ apart, since the Ti–Ti and Ti–S vectors give the most pronounced maxima in the Patterson projections. The arrangement is possible only if $y_{Ti} \approx \frac{1}{4}$ and $y_S \approx \frac{1}{4}$ or $\frac{3}{4}$.

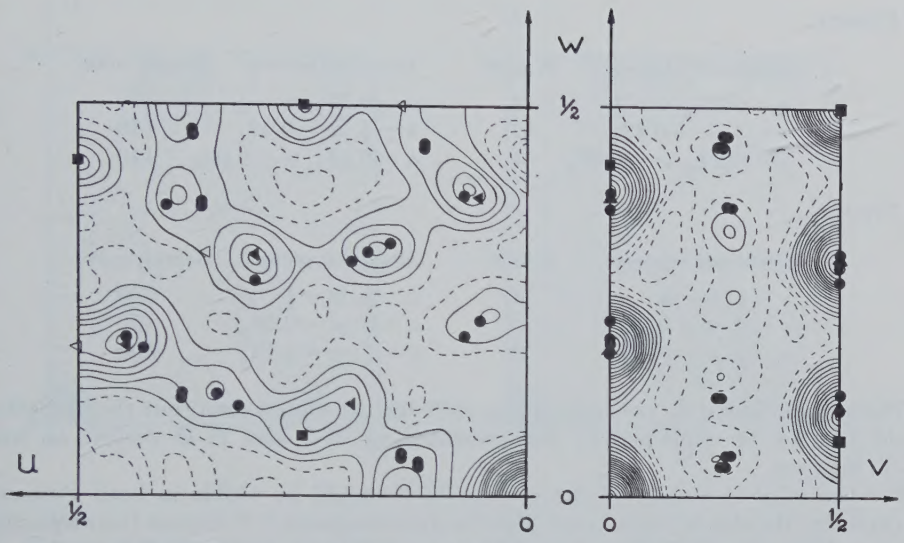

Fig. 1. The Patterson projections $P(upw)$ and $P(pvw)$ for $TiOSO_4H_2O$. The Ti–Ti (■), Ti–S (▲), Ti–O (●), and S–S (△) vectors have been marked for the final idealized structure. Dashed lines indicate negative values.

The x and z parameters of the titanium and sulphur atoms can be obtained from $P(upw)$. In this projection the Ti–Ti vectors are:

I.	$u = \frac{1}{2} - 2x_{Ti}$, $w = \frac{1}{2}$	weight = 2
II.	$u = \frac{1}{2}$, $w = 2z_{Ti}$	,, = 2
III.	$u = 2x_{Ti}$, $w = \frac{1}{2} - 2z_{Ti}$	,, = 1

I and II should be clearly seen on the lines $w = \frac{1}{2}$ and $u = \frac{1}{2}$ in $P(upw)$ and we could thus identify I as $\pm(0.13_3, \frac{1}{2})$; $\pm(0.25_0, \frac{1}{2})$; or $\pm(0.39_2, \frac{1}{2})$ and II as $(\frac{1}{2}, \pm 0.19_2)$ or $(\frac{1}{2}, \pm 0.42_4)$, which gives the following possible values for x_{Ti} and z_{Ti} (we need only consider the range $0 \leq x_{Ti} \leq \frac{1}{4}$; $0 \leq y_{Ti} \leq \frac{1}{4}$; $0 \leq z_{Ti} \leq \frac{1}{4}$):

$$x_{Ti} = 0.05_4;\ 0.12_5;\ 0.18_4$$
$$z_{Ti} = 0.09_6;\ 0.21_2$$

The parameters were then combined and tested by calculating the third Ti–Ti vector (III), which should also be clearly seen in $P(upw)$. In this way all pairs were ruled out except one. The parameters of the titanium atoms should then be:

$$4\ \text{Ti in 4(a) with } x \approx 0.12_5,\ y \approx 0.25,\ z \approx 0.21_2.$$

The titanium atoms are thus arranged in zig-zag chains, which are parallel to the b-axis.

The Ti–Ti vectors calculated with these parameters are:

In $P(upw)$:

Calculated vectors	Weight	Observed vectors	Height, units
$u = 0.25_0$, $w = \frac{1}{2}$	2	$u = 0.25_0$, $w = \frac{1}{2}$	150
$u = \frac{1}{2}$, $w = 0.42_4$	2	$u = \frac{1}{2}$, $w = 0.42_4$	140
$u = 0.25_0$, $w = 0.07_6$	1	$u = 0.23_7$, $w = 0.09_5$	140

In $P(pvw)$:

Calculated vectors	Weight	Observed vectors	Height, units
$v = \frac{1}{2}$, $w = \frac{1}{2}$	4	$v = \frac{1}{2}$, $w = \frac{1}{2}$	650
$v = 0$, $w = 0.42_4$	2	$v = 0$, $w = 0.39_5$	450
$v = \frac{1}{2}$, $w = 0.07_6$	2	$v = \frac{1}{2}$, $w = 0.10_0$	500

The weights found do not quite agree with the calculated data, but the deviations could later on be explained by superpositions of Ti–S and Ti–O vectors on some Ti–Ti maxima.

For the sulphur atoms, all the vectors Ti–S should be visible in both Patterson projections. We should also expect that the most frequent S–S vectors (corresponding to the vectors I and II above) should appear in $P(upw)$ and from them we get the following possible parameters for the sulphur atoms. (Here we must consider the range $0 \leq x_S \leq \frac{1}{2}$; $0 \leq z_S \leq \frac{1}{2}$, if the limits for y_S are $0 \leq y_S \leq 1$.)

$$x_S = 0.05_4;\ 0.12_5;\ 0.18_4;\ 0.31_7;\ 0.37_5;\ 0.44_6$$
$$z_S = 0.09_6;\ 0.21_2;\ 0.28_8;\ 0.40_4;$$

The parameters were then combined and tested by calculating the Ti–S vectors in $P(upw)$. We found then that only the following pairs could be possible:

$$\begin{cases} x_S = 0.31_7 \\ z_S = 0.09_6 \end{cases} \qquad \begin{cases} x_S = 0.44_6 \\ z_S = 0.09_6 \end{cases} \qquad \begin{cases} x_S = 0.12_5 \\ z_S = 0.21_2 \end{cases}$$

Each of these possibilities must be combined with $y_S = 0.25$ or $y_S = 0.75$ giving six alternatives for the sulphur parameters. They were then tested by calculating the Ti–S vectors in $P(pvw)$. Agreement between observed and calculated data were then obtained only with the following sets of parameters:

A.	B.	C.
$x_S = 0.31_7$	$x_S = 0.44_6$	$x_S = 0.12_5$
$y_S = 0.75$	$y_S = 0.75$	$y_S = 0.25$
$z_S = 0.09_6$	$z_S = 0.09_6$	$z_S = 0.21_2$

The last set C could at once be ruled out as possible sulphur parameters since it coincides with the titanium positions in the unit cell. The first two sets, however, seem to be about equally plausible when compared with $P(upw)$ and $P(pvw)$, even if the agreement might be a little better for A than for B. Nor is it possible to decide between the two cases by calculating $P(uvp)$ since they both give nearly the same maxima in this projection.

By introducing three-dimensional Patterson calculations, it should however be possible to decide which of the two sets A and B gives the correct sulphur position in the unit cell. Now, as shown by $P(pvw)$, most of the interatomic vectors have the

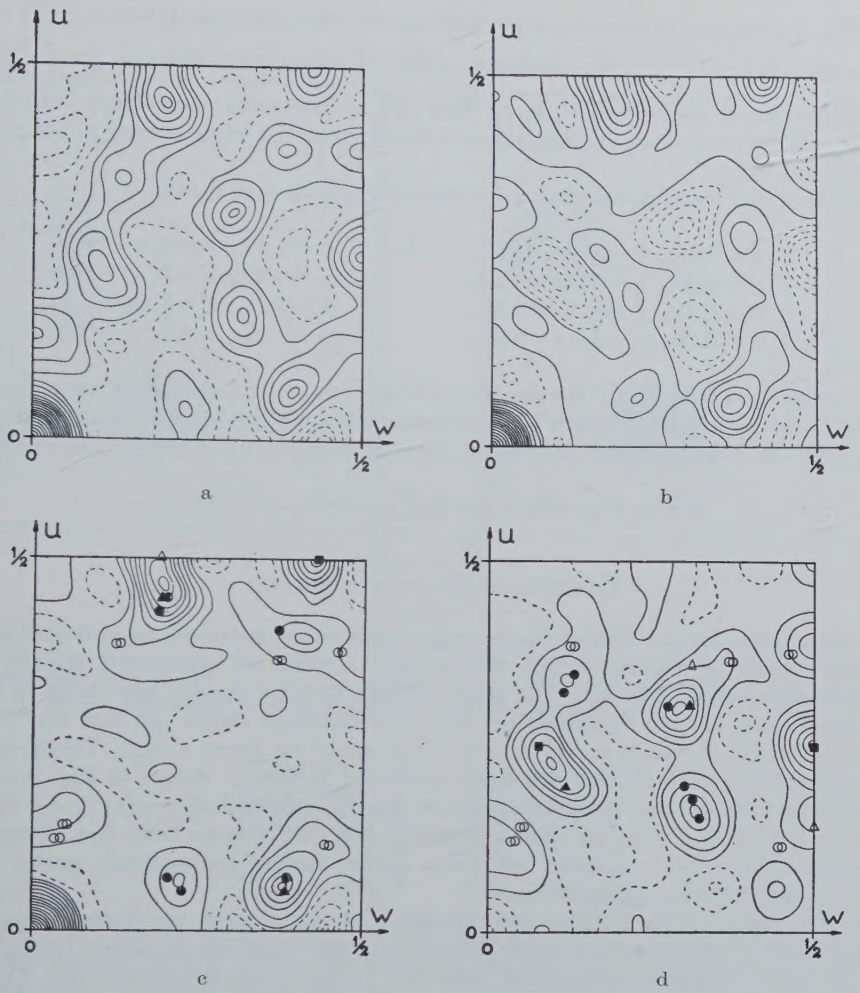

Fig. 2. The functions (a) $\sum_h \sum_l |F|^2_{h0l} \cos 2\pi(hu+lw)$ (upper left)
and (b) $\sum_h \sum_l |F|^2_{h1l} \cos 2\pi(hu+lw)$ (upper right).

The approximate sections through the Patterson space for (c) $v = 0$ (lower left) and (d) $v = \frac{1}{2}$ (lower right) are also shown. The vectors for the final idealized structure have been marked as in Fig. 1. Dashed lines indicate negative values.

v coordinates 0 or $\frac{1}{2}$, which implies not only that y_{Tl} and y_{S} are $\frac{1}{4}$ and $\frac{3}{4}$ but also that some of the oxygen atoms should be situated in, or close to, the planes $y = \pm\frac{1}{4}$. In that case the following *approximate* relations hold:

$$P(u0w) \approx \sum_h \sum_l (|F|^2_{h0l} + |F|^2_{h1l}) \cos 2\pi(hu+lw)$$

$$P(u\tfrac{1}{2}w) \approx \sum_h \sum_l (|F|^2_{h0l} - |F|^2_{h1l}) \cos 2\pi(hu+lw)$$

In Fig. 2 these two functions are plotted together with their partial sums $\sum_h \sum_l |F|^2_{h0l} \cos 2\pi(hu + lw)$ and $\sum_h \sum_l |F|^2_{h1l} \cos 2\pi(hu + lw)$, of which the latter also gives a generalized Patterson projection $P_1(x, z)$. The $|F|$ values were normalized before the calculations by comparing corresponding reflections in the Weissenberg photographs *h0l*, *h1l*, and *0kl*.

Now, the Ti–S vectors calculated for the two sets A and B are:

A.	B.
1. $u = 0.05_8$, $v = 0$, $w = 0.38_4$	1. $u = 0.07_1$, $v = 0$, $w = 0.38_4$
2. $u = 0.44_2$, $v = 0$, $w = 0.19_2$	2. $u = 0.42_9$, $v = 0$, $w = 0.19_2$
3. $u = 0.19_2$, $v = \frac{1}{2}$, $w = 0.11_6$	3. $u = 0.32_1$, $v = \frac{1}{2}$, $w = 0.11_6$
4. $u = 0.30_8$, $v = \frac{1}{2}$, $w = 0.30_8$	4. $u = 0.17_9$, $v = \frac{1}{2}$, $w = 0.30_8$

By comparison with Fig. 2 we see that all the vectors in A come out very clearly, but for B the vector B3 is much less pronounced than expected. We may thus conclude that the sulphur atoms should be situated as given by A, i.e. in the following way:

$$4\ \text{S in } 4(a) \text{ with } x \approx 0.31_7,\ y \approx 0.75,\ z \approx 0.09_6.$$

Positions of the oxygen atoms

To determine the positions of the oxygen atoms in the unit cell, we calculated the electron density projection $\varrho(xpz)$, which has a centre of symmetry, though the structure as a whole has not. The signs of the structure factors were then at first determined only by the contributions from the titanium atoms. In this way 24 of the 49 observed F values in *h0l* ($h \geq 0$, $l \geq 0$) could be given a sign. The electron density projection calculated with these structure factors is shown in Fig. 3a. We see that the titanium and sulphur atoms are clearly indicated at $x = 0.12_5$, $z = 0.21_0$, and at $x = 0.32_5$, $z = 0.09_2$ in close agreement with the results from the Patterson calculations. In addition, there are some other, rather pronounced, peaks that should correspond to oxygen atoms.

The next step in the Fourier determination of the oxygen parameters was the calculation of a new electron density projection $\varrho(xpz)$ from the structure factors the signs of which could be determined from the Ti and S contributions only. Of the 49 observed reflections, 41 could then be given a sign. The results are shown in Fig. 3b and we see that all except one of the "oxygen" peaks from Fig. 3a have appeared again. The x and z parameters of the oxygen atoms in the unit cell should then be:

A.	$x = 0.02_5$	$z = 0.28_3$
B.	$x = 0.44_2$	$z = 0.10_0$
C.	$x = 0.23_0$	$z = 0.41_3$
D.	$x = 024_3$	$z = 0.16_2$
E.	$x = 0.42_5$	$z = 0.48_3$

The parameters were then checked against $P(upw)$ by calculating the Ti–O vectors, all of which should be seen in this Patterson projection. It was then found that they all gave a rather good agreement with the observed peaks in $P(upw)$ (cf. Fig. 1).

The distances from the oxygen atoms to their nearest titanium and sulphur neighbours in the *xz* projection are:

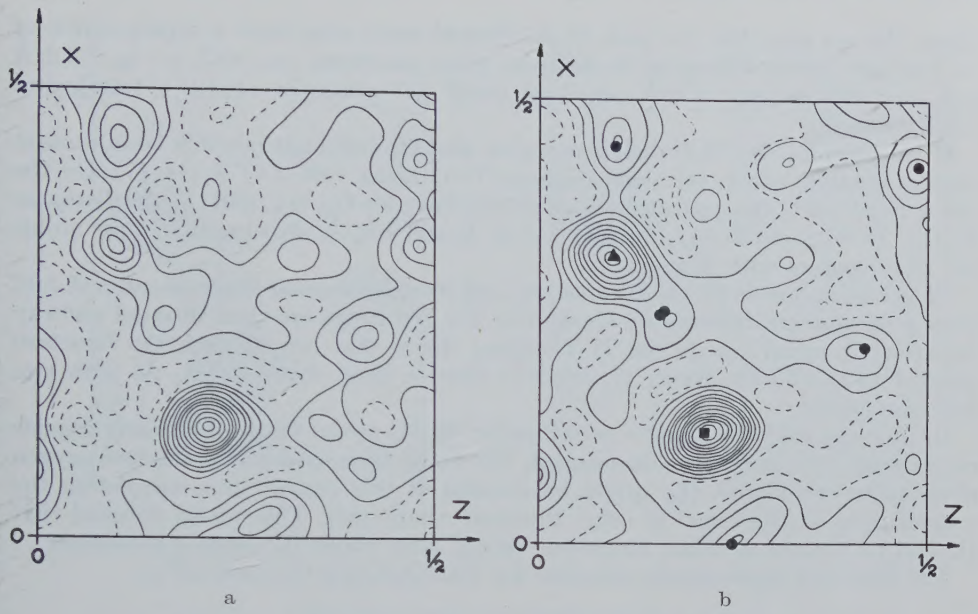

Fig. 3. The electron density projection $\varrho(xpz)$ calculated with the F values for which the signs were determined by (a) the titanium contributions only (left) and (b) the titanium and sulphur contributions only (right). The final idealized positions of the titanium (■), sulphur (▲), and oxygen (●) atoms have been marked. Dashed lines indicate negative values.

$Ti–O_A = 1.2_7$ Å	$S–O_A = 3.3_8$ Å
$Ti–O_B = 3.2_0$ Å	$S–O_B = 1.2_7$ Å
$Ti–O_C = 1.9_5$ Å	$S–O_C = 1.6_2$ Å
$Ti–O_D = 1.3_0$ Å	$S–O_D = 0.8_7$ Å
$Ti–O_E = 1.9_9$ Å	$S–O_E = 2.5_0$ Å

We see at once from this table that O_A and O_E should correspond to the non-sulphate oxygen atoms in the unit cell since the distances from their neighbouring sulphur atoms are much longer than the S–O distance within a sulphate group (about 1.4_5 Å (13)). O_B, O_C, and O_D should consequently be sulphate oxygen atoms.

The y parameters of the oxygen atoms might be obtained from $P(pvw)$ (Fig. 1) or $\varrho(pyz)$ calculated from the structure factors for which the signs could be determined from the titanium and sulphur contributions. We would, however, expect that in these projections the oxygen peaks should be superimposed because of the rather long x and z axes and the short y axis. We would also expect that the projections should be somewhat deformed owing to irregular absorption in the crystals when rotated about [100]. For these reasons it was concluded that approximate y parameters are most easily found by considerations of the S–O and Ti–O distances in the crystal.

For the sulphate oxygen atoms we should expect a shortest S–O distance of about 1.45 Å (13). We see then that O_B and O_C above should have about the same y parameter as their closest sulphur neighbours whereas the y parameter of O_D should differ by about 1.2 Å (i.e. a little less than $b/4$) from that of its nearest sulphur

atom. We see also that the peak O_D in $P(upw)$ must arise from a superposition of two oxygen atoms belonging to different point positions, one with $y = y_S + 1.2$ Å and one with $y = y_S - 1.2$ Å, since we could not otherwise obtain a tetrahedral sulphate group.

If we consider the Ti–O distances we see that O_B could not possibly be in contact with a titanium atom, the bond distance Ti–O being 1.86–2.17 Å (14–17). On the other hand with the y parameters obtained above for O_C, O_{D_1}, and O_{D_2} the distances Ti–O_C, Ti–O_{D_1}, and Ti–O_{D_2} are 1.9_5 Å, 1.8_3 Å, and 1.8_3 Å, showing that these atoms are all in contact with titanium.

To get a reasonable Ti–O_A distance, O_A and its neighbouring titanium atom should have y parameters differing by about $b/4$. The O_A atoms are then situated midway between the metal ions in the Ti–Ti zigzag chains that run through the structure parallel to the b-axis. Every O_A atom is thus in close contact (1.8_0 Å) with two titanium atoms.

In this way we have found a coordination Ti–5O in the shape of a nearly regular octahedron with one corner unoccupied. We would then expect that the last oxygen atom to be considered, O_E, would be situated at this corner, thus completing the coordination Ti–6O found in other titanium compounds. This would demand that O_E and its nearest titanium neighbour should have about the same y parameter.

The following approximate structure for $TiOSO_4H_2O$ is thus arrived at:

4 Ti in $P2_12_12_1$	4(a)	with	$x = 0.12_7$,	$y = 0.25$,	$z = 0.21_3$
4 S	,,	,,	,,	,,	$x = 0.32_2$,	$y = 0.75$,	$z = 0.09_4$
4 O_1	,,	,,	,,	,,	$x = 0.00$,	$y = 0.00$,	$z = 0.25$
4 O_2	,,	,,	,,	,,	$x = 0.44_7$,	$y = 0.75$	$z = 0.10_0$
4 O_3	,,	,,	,,	,,	$x = 0.21_6$,	$y = 0.25$,	$z = 0.41_4$
4 O_4	,,	,,	,,	,,	$x = 0.25$	$y = 0.51$,	$z = 0.17$
4 O_5	,,	,,	,,	,,	$x = 0.25$,	$y = 0.99$,	$z = 0.17$
4 O_6	,,	,,	,,	,,	$x = 0.41_9$,	$y = 0.75$,	$z = 0.48_6$

This set of parameters was then checked against the Patterson projections $P(upw)$ and $P(pvw)$ by calculating the Ti–Ti, Ti–S, Ti–O, and S–S vectors. As is seen in Fig. 1, the agreement between observed and calculated data is satisfactory.

The eight hydrogen atoms in the unit cell should be situated close to some of the oxygen atoms and should then certainly belong to the non-sulphate oxygen atoms O_1 or O_6 since the proton affinity is much larger for O^{2-} than for SO_4^{2-}. They could be distributed over both these positions but they could also belong to either O_1 or O_6. However, every O_1 is in close contact with two titanium atoms whereas every O_6 is in close contact with only one titanium and thus it seems to be most reasonable that the hydrogen atoms are all attached to O_6. The position O_1 should then consist of O^{2-} ions and the position O_6 of H_2O molecules. The hydrogen atoms surely form hydrogen bonds to neighbouring oxygen atoms.

Refinement of the parameters

The parameters of the preliminary structure above were then refined. The x and z parameters were determined from the xz projection of the electron density (with all the observed reflections introduced in the calculations). To eliminate the diffrac-

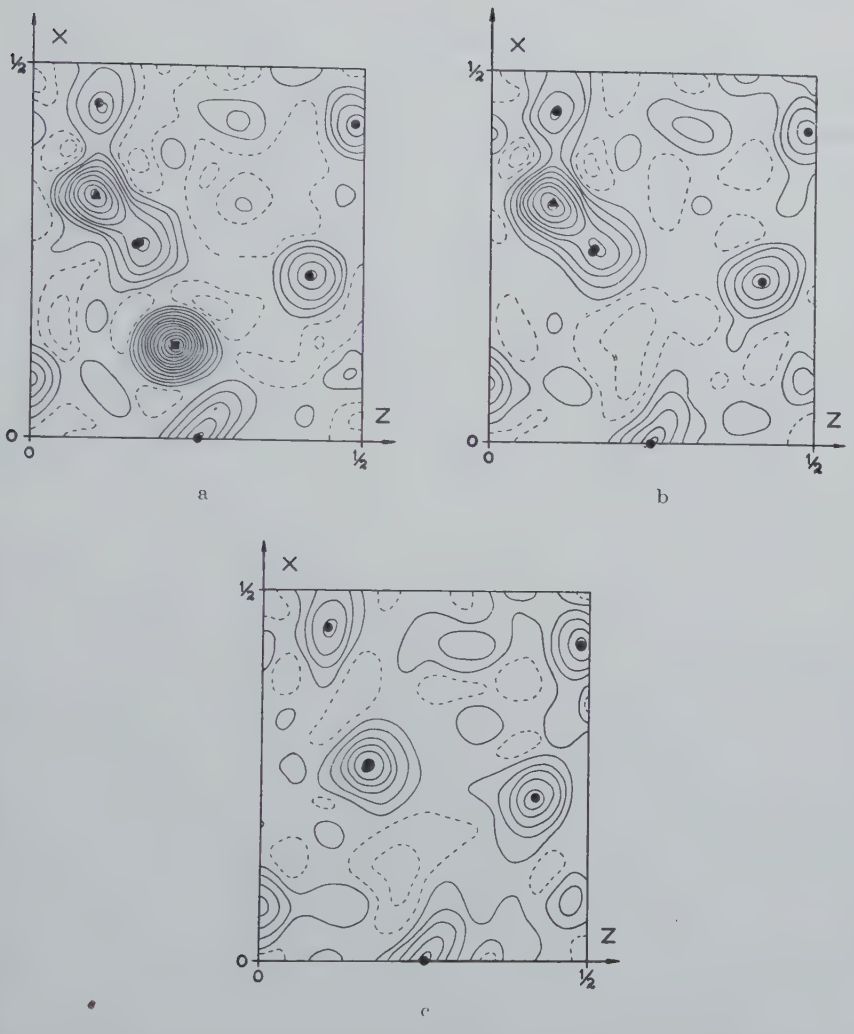

Fig. 4. The final electron density projection $\varrho(xpz)$ of $TiOSO_4H_2O$ (upper left). The figure shows also the electron density projections $\varrho(xpz)$ with (b) the titanium atoms (upper right) and (c) the titanium and sulphur atoms (below) subtracted. The positions of the atoms have been marked as in Fig. 3. Dashed lines indicate negative values.

tion effects as much as possible the titanium and sulphur atoms were also subtracted from the function (cf. Fig. 4). The structure factors were then corrected by an exponential factor of $10^{-0.28 \sin^2 \theta}$, obtained in the ordinary way for the determination of temperature factors. In this way, rather reliable x and z values could be obtained for Ti, S, O_2, O_3, and O_6. The parameters of O_1, O_4, and O_5 could however not be determined with the corresponding degree of accuracy since they are superimposed in $\varrho(xpz)$.

Table 2. Calculated and observed structure factors in Weissenberg photographs of $TiOSO_4H_2O$.

h k l	*F* calc	*F* obs	*h k l*	*F* calc	*F* obs	*h k l*	*F* calc	*F* obs	*h k l*	*F* calc	*F* obs
2 0 0	−39	22	7 0 4	−43	37	2 1 0	−125	94	1 1 4	14	18
4 0 0	5	0	8 0 4	23	20	3 1 0	0	0	2 1 4	−10	15
6 0 0	−7	20	9 0 4	22	19	4 1 0	12	12	3 1 4	−13	8
8 0 0	17	0	10 0 4	8	0	5 1 0	0	0	4 1 4	−23	11
10 0 0	−4	0	11 0 4	7	16	6 1 0	1	0	5 1 4	−26	35
12 0 0	−2	0				7 1 0	0	0	6 1 4	30	30
			1 0 5	18	10	8 1 0	−1	0	7 1 4	10	0
1 0 1	44	30	2 0 5	70	64	9 1 0	0	0	8 1 4	7	0
2 0 1	−17	13	3 0 5	−4	0	10 1 0	−5	0	9 1 4	26	35
3 0 1	−3	8	4 0 5	−9	15	11 1 0	0	0	10 1 4	−14	0
4 0 1	42	41	5 0 5	7	0	12 1 0	−1	0	11 1 4	−17	18
5 0 1	−29	28	6 0 5	−4	0						
6 0 1	−34	32	7 0 5	7	0	0 1 1	−38	38	0 1 5	88	81
7 0 1	37	27	8 0 5	4	0	1 1 1	−28	14	1 1 5	−9	0
8 0 1	−3	0	9 0 5	18	15	2 1 1	37	26	2 1 5	−9	0
9 0 1	31	32				3 1 1	−21	21	3 1 5	−3	0
10 0 1	5	0	0 0 6	−27	29	4 1 1	−22	26	4 1 5	−28	38
11 0 1	−11	23	1 0 6	−5	0	5 1 1	21	28	5 1 5	7	0
12 0 1	−3	0	2 0 6	−5	0	6 1 1	−19	30	6 1 5	6	20
			3 0 6	−19	0	7 1 1	38	33	7 1 5	9	0
0 0 2	−39	23	4 0 6	7	0	8 1 1	18	19	8 1 5	−3	0
1 0 2	−80	51	5 0 6	22	27	9 1 1	−22	0	9 1 5	−7	0
2 0 2	−4	9	6 0 6	−30	27	10 1 1	−1	0	10 1 5	0	0
3 0 2	−2	0	7 0 6	21	23	11 1 1	−15	18			
4 0 2	15	18	8 0 6	10	0	12 1 1	−3	0	0 1 6	0	0
5 0 2	3	17	9 0 6	−15	16				1 1 6	−9	0
6 0 2	−6	0				0 1 2	0	9	2 1 6	22	29
7 0 2	−5	0	1 0 7	3	0	1 1 2	−44	29	3 1 6	14	30
8 0 2	−49	40	2 0 7	−12	0	2 1 2	14	0	4 1 6	−22	35
9 0 2	−7	0	3 0 7	−17	0	3 1 2	30	25	5 1 6	22	14
10 0 2	−2	0	4 0 7	−3	0	4 1 2	−2	0	6 1 6	10	0
11 0 2	5	0	5 0 7	2	0	5 1 2	0	0	7 1 6	−20	0
12 0 2	10	20	6 0 7	30	31	6 1 2	−44	41	8 1 6	4	0
						7 1 2	−11	33	9 1 6	−18	19
1 0 3	−79	53	0 0 8	1	0	8 1 2	2	0			
2 0 3	−6	8	1 0 8	19	29	9 1 2	13	0	0 1 7	−3	0
3 0 3	24	24	2 0 8	13	20	10 1 2	25	29	1 1 7	−14	0
4 0 3	8	0	3 0 8	33	33	11 1 2	−5	0	2 1 7	−6	0
5 0 3	0	0	4 0 8	6	0				3 1 7	0	0
6 0 3	36	35	5 0 8	−27	30	0 1 3	−1	0	4 1 7	24	20
7 0 3	−21	31				1 1 3	46	31	5 1 7	8	0
8 0 3	−6	0	1 0 9	2	0	2 1 3	6	17	6 1 7	1	0
9 0 3	5	0	2 0 9	12	0	3 1 3	42	34	7 1 7	−15	0
10 0 3	−14	0	3 0 9	4	0	4 1 3	22	31	8 1 7	−27	36
11 0 3	−6	0	4 0 9	5	0	5 1 3	−28	34			
						6 1 3	−5	0	0 1 8	0	0
0 0 4	−4	11	0 0 10	21	28	7 1 3	12	0	1 1 8	30	38
1 0 4	40	31	1 0 10	−3	0	8 1 3	−25	0	2 1 8	2	0
2 0 4	49	33	2 0 10	2	0	9 1 3	1	0	3 1 8	−25	33
3 0 4	−3	13	3 0 10	−5	0	10 1 3	−1	0	4 1 8	−6	0
4 0 4	−30	33	4 0 10	−18	21	11 1 3	3	0	5 1 8	−7	0
5 0 4	6	0							6 1 8	−6	0
6 0 4	1	17	1 1 0	0	9	0 1 4	0	0	7 1 8	10	14

Table 2 (*continued*)

h k l	*F* calc	*F* obs	*h k l*	*F* calc	*F* obs	*h k l*	*F* calc	*F* obs	*h k l*	*F* calc	*F* obs
0 1 9	9	16	0 2 6	38	30	1 4 6	−4	0	3 5 0	0	0
1 1 9	1	0	0 3 6	0	19	1 4 7	2	0	4 5 0	9	0
2 1 9	4	0	0 4 6	−17	25				5 5 0	0	0
3 1 9	6	0	0 5 6	0	17	1 5 0	0	28	6 5 0	1	0
4 1 9	−14	0				1 5 1	−6	0			
5 1 9	2	0	0 2 7	0	0	1 5 2	−16	21	2 6 0	3	0
			0 3 7	1	0	1 5 3	22	21	3 6 0	0	0
0 1 10	0	0	0 4 7	0	0	1 5 4	6	0			
1 1 10	−5	0				1 5 5	−7	0	2 2 1	8	10
2 1 10	24	22	0 2 8	5	19	1 5 6	−6	0	3 2 1	39	36
3 1 10	7	0	0 3 8	0	0				4 2 1	−28	27
						1 6 0	0	0	5 2 1	35	27
0 2 0	−84	92	0 2 9	0	0	1 6 1	−3	0	6 2 1	21	18
0 4 0	98	84				1 6 2	7	21	7 2 1	−7	0
0 6 0	−30	34	0 2 10	40	39	1 6 3	13	19	8 2 1	9	11
									9 2 1	−27	26
			1 2 0	0	0	2 2 0	−2	0	10 2 1	−11	0
0 2 1	0	25	1 2 1	−3	6	3 2 0	0	21	11 2 1	25	24
0 3 1	19	20	1 2 2	5	0	4 2 0	71	54			
0 4 1	0	16	1 2 3	34	34	5 2 0	0	0	2 3 1	−20	23
0 5 1	−13	17	1 2 4	2	0	6 2 0	−12	0	3 3 1	14	0
0 6 1	0	14	1 2 5	6	16	7 2 0	0	0	4 3 1	11	0
			1 2 6	13	20	8 2 0	17	0	5 3 1	−13	0
0 2 2	−26	26	1 2 7	−16	0	9 2 0	0	0	6 3 1	15	28
0 3 2	0	0	1 2 8	−39	36	10 2 0	−2	0	7 3 1	−31	28
0 4 2	−18	0	1 2 9	7	0	11 2 0	0	0	8 3 1	−16	22
0 5 2	0	19	1 2 10	10	0				9 3 1	19	30
0 6 2	−3	0				2 3 0	74	79			
			1 3 0	0	0	3 3 0	0	0	2 4 1	−8	18
0 2 3	0	0	1 3 1	19	26	4 3 0	−10	0	3 4 1	−3	0
0 3 3	8	19	1 3 2	28	28	5 3 0	0	0	4 4 1	21	28
0 4 3	0	24	1 3 3	−33	38	6 3 0	−4	0	5 4 1	−18	16
0 5 3	−1	0	1 3 4	−10	20	7 3 0	0	0	6 4 1	−18	0
0 6 3	0	0	1 3 5	5	0	8 3 0	2	0	7 4 1	11	0
			1 3 6	8	11	9 3 0	0	0			
0 2 4	−3	0	1 3 7	12	0				2 5 1	14	0
0 3 4	0	0	1 3 8	−25	38	2 4 0	−16	0	3 5 1	−15	0
0 4 4	−1	0				3 4 0	0	0	4 5 1	−9	0
0 5 4	0	0	1 4 0	0	16	4 4 0	−6	0	5 5 1	12	0
			1 4 1	18	17	5 4 0	0	0	6 5 1	−9	0
0 2 5	0	0	1 4 2	−34	34	6 4 0	1	0			
0 3 5	−66	63	1 4 3	−36	34	7 4 0	0	0	2 6 1	4	0
0 4 5	0	0	1 4 4	17	0				3 6 1	11	0
0 5 5	39	36	1 4 5	10	0	2 5 0	−44	41	4 6 1	−11	0

The structure arrived at in this way corresponds to the pseudo symmetry of the crystals according to the space group no. 62 *Pnma* (11). If the signs of the structure factors $0kl$ with $k + l = 2n$ are obtained from the approximate structure above and also those of the few observed structure factors $0kl$ with $k + l = 2n + 1$ by some other method, then the deviations from *Pnma* can be obtained from calculations of $\varrho(pyz)$. A three-dimensional Patterson calculation would perhaps also solve the problem. However, during several attempts to solve the crystal structure in this way, it be-

came obvious that the experimental intensity material was too poor to allow a complete structure determination. Attempts were then made to grow bigger crystals that could give better Weissenberg photographs but, as pointed out earlier, they were all in vain.

A last attempt to solve the structure by trial and error methods assuming that the deviations from *Pnma* were due to the displacement of some of the oxygen atoms only (for example O_1, the peak of which is rather elongated in $\varrho(xpz)$) was also without success.

We must thus restrict our conclusions to the following idealized structure:

Space group: no. 19 $P2_12_12_1$. Four formula units in the unit cell.

Atomic positions:

4 Ti	in	4(a)	with	$x = 0.125$,	$y = 0.25$, $z = 0.213$
4 S	,,	,,	,,	$x = 0.321$,	$y = 0.75$, $z = 0.095$
4 O_1	,,	,,	,,	$x = 0.00$,	$y = 0.00$, $z = 0.25$
4 O_2	,,	,,	,,	$x = 0.447$,	$y = 0.75$, $z = 0.099$
4 O_3	,,	,,	,,	$x = 0.221$,	$y = 0.25$, $z = 0.418$
4 O_4	,,	,,	,,	$x = 0.26$,	$y = 0.51$, $z = 0.16$
4 O_5	,,	,,	,,	$x = 0.26$,	$y = 0.99$, $z = 0.16$
4 $O_6(H_2O)$	,,	,,	,,	$x = 0.428$,	$y = 0.75$, $z = 0.487$

4(a): x,y,z;; $\frac{1}{2}-x,\bar{y},\frac{1}{2}+z$; $\frac{1}{2}+x,\frac{1}{2}-y,\bar{z}$; $\bar{x},\frac{1}{2}+y,\frac{1}{2}-z$.

Parameter values printed in italics are considered to be more accurate (estimated accuracy ± 0.005) than the others.

The structure factors calculated with these parameter values and corrected with the exponential factor $10^{-0.28\sin^2\theta}$ (cf. above) are compared with the observed ones in Table 2. As is seen, the agreement between calculated and observed data is fairly good, considering that the structure is only approximate. Because of the shape of the crystals the Weissenberg photographs $hk0$, $hk1$, $0kl$, and $1kl$ show absorption effects (cf. Wells (18)).

Description of the structure

The structure of $TiOSO_4H_2O$ contains chains of titanium and oxygen atoms (Fig. 5). They are planar in the idealized structure but the fact that the O_1 peak in $\varrho(xpz)$ is rather elongated suggests that they are screwlike in the real structure. Nor is it certain that the two groups of Ti–O distances in the chains have the same lengths.

The chains are joined to each other by sulphate groups each of which is in contact with three metal ions. The water molecules are situated close to titanium atoms so that a coordination Ti–6O in the shape of an octahedron is obtained. A schematic drawing of the structure is shown in Fig. 6.

The shortest distances Ti–Ti and Ti–O for the idealized structure are:

Ti–Ti = 3.60 Å	Ti–O_4 = 1.9_3 Å
Ti–2O_1 = 1.8_0 Å	Ti–O_5 = 1.9_3 Å
Ti–O_3 = 2.0_0 Å	Ti–O_6 = 2.0_1 Å

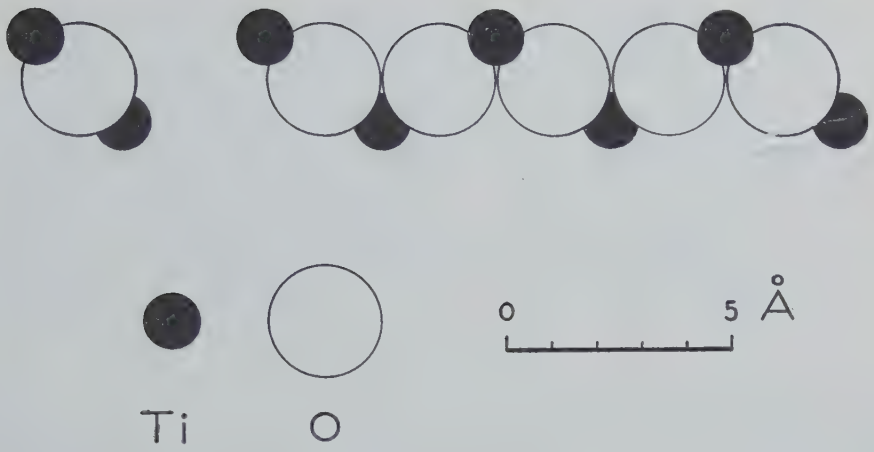

Fig. 5. The idealized Ti–O chain, viewed along the chain (left) and perpendicular to it (right).

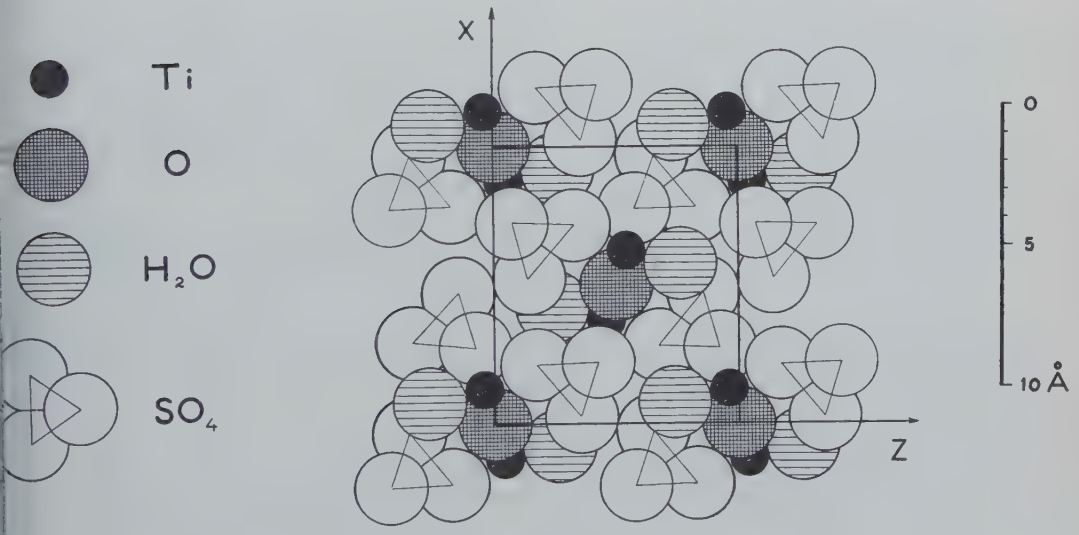

Fig. 6. The idealized structure of $TiOSO_4H_2O$, viewed along [010].

This is in good agreement with the corresponding figures of 1.91–1.98 Å and 1.86–2.17 Å found in TiO_2 (14, 15) and the titanates (16, 17). However, because of the low accuracy in the determination of the atomic parameters, we could not say anything about the bond strength or bond order of the Ti–O bonds in $TiOSO_4H_2O$.

The S–O and O–O distances in the structure are:

$S–O_2 = (1.2_4$ Å)
$S–O_3 = (1.5_7$ Å)
$S–O_4 = (1.4_7$ Å)
$S–O_5 = (1.4_7$ Å)
$O_1–2O_1 = 2.5_6$ Å
$O_1–2O_2$; $O_2–2O_1 = 3.3_0$ Å
$O_1–2O_3$; $O_3–2O_1 = 2.1_0$ Å
$O_1–O_4$; $O_4–O_1 = 2.6_6$ Å
$O_1–O_5$; $O_5–O_1 = 2.6_6$ Å
$O_1–2O_6$; $O_6–2O_1 = 2.6_9$ Å
$O_2–2O_2 = 3.0_8$ Å
$O_2–2O_3$; $O_3–2O_2 = 2.2_6$ Å*; 3.2_5 Å
$O_2–O_4$; $O_4–O_2 = 2.2_6$ Å*
$O_2–O_5$; $O_5–O_2 = 2.2_6$ Å*
$O_2–2O_6$; $O_6–2O_2 = 2.9_3$Å; 3.3_4 Å
$O_3–2O_4$; $O_4–2O_3 = 2.4_2$ Å;* 2.6_2 Å
$O_3–2O_5$; $O_5–2O_3 = 2.4_2$ Å*; 2.6_2 Å
$O_3–2O_6$; $O_6–2O_3 = 3.3_2$ Å
$O_4–2O_5$; $O_5–2O_4 = 2.4_6$ Å*; 2.6_6 Å
$O_4–O_6$; $O_6–O_4 = 2.7_1$ Å
$O_5–O_6$; $O_6–O_5 = 2.7_1$ Å

O–O distances within a sulphate group have been marked with an asterisk.

The S–O distances are given in brackets since they were used for the determination of the oxygen y parameters. The O–O distances are all within the normal range showing that the structure is also supported by O–O contacts. The accuracy of the O–O distances is, however, $\pm 0.1-0.2$ Å.

Summary

The crystals of $TiOSO_4H_2O$ are orthorhombic with $a = 9.788 \pm 0.005$ Å, $b = 5.120 \pm 0.003$ Å, and $c = 8.598 \pm 0.005$ Å. They belong to the space group no. 19 $P2_12_12_1$ but show pseudo-symmetry according to no. 62 $Pnma$ or no. 33 $Pn2_1a$. The atomic parameters were determined by Patterson and Fourier methods considering the real space group $P2_12_12_1$. The arrangement arrived at (cf. page 410) also fits in with $Pnma$ and may be considered as an idealized structure which should be slightly distorted to give the real symmetry according to $P2_12_12_1$.

The structure is built up of $(TiO)_n^{2n-}$ chains, which are joined to each other by sulphate groups. The water molecules are situated close to the titanium atoms. The coordination Ti–O is sixfold in the shape of a somewhat deformed octahedron.

The author wishes to thank Professor Lars Gunnar Sillén and Dr. Arne Magnéli for valuable discussions during this work. He is also indebted to Professor Arne Ölander and Professor Gustaf Ljunggren for their kind interest.

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REFERENCES

1. GMELIN's Handbuch, 8. Aufl., *41* Titan. Verlag Chemie, Weinheim/Bergstrasse, 1951, pp. 348–349.
2. LUNDGREN, G. and SILLÉN, L. G., Arkiv för Kemi *1* (1949) 277.
3. LUNDGREN, G., ibid. *2* (1950) 535.
4. LUNDGREN, G., ibid. *4* (1952) 421.
5. LUNDGREN, G., ibid. *5* (1953) 349.
6. LUNDGREN, G., ibid. *6* (1953) 59.

7. LUNDGREN, G., ibid., *10* (1956) 183.
8. PAMFILOV, A. V. and KHUDYAKOVA, T. A., Zhur. Obschei Khim. *19* (1949) 443 as given in C.A. *44* (1950) 1354[a].
9. KOLTHOFF, I. M. and SANDELL, E. B., Textbook of Quantitative Inorganic Analysis. New York, 1943, p. 753.
10. HARTWIG-BENDIG, M., Z. angew. Miner. *3* (1941) 195.
11. International Tables for X-ray Crystallography, Vol I. The Kynoch Press, Birmingham, 1952.
12. LU, C. S., Rev. Sci. Instr. *14* (1943) 331.
13. WELLS, A. F., J. Chem. Soc. *1949*, 55.
14. BAUR, W. H., Acta Cryst. *9* (1956) 515.
15. CROMER, D. T. and HERRINGTON, K., J. Am. Chem. Soc. *77* (1955) 4708.
16. EVANS, H. T., Acta Cryst. *4* (1951) 377.
17. MEGAW, H., ibid *5* (1952) 739.
18. WELLS, A. F., Z. Krist. *96* (1937) 451.

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Communicated 6 June 1956 by Einar Hammarsten and Erik Jorpes

Purification of human and bovine fibrinogen

By Birger Blombäck and Margareta Blombäck

With 4 figures in the text

Historical

In the middle of the 17th century, Malpighi described fibrin as a white fibrinous substance obtainable from the red blood clot through washing with water.

While it had formerly been widely believed that coagulation occurred through some kind of aggregation of the red blood cells, Hewson showed in about 1770 that the clotting process is independent of the formed elements and takes place in the blood lymph (plasma). Like Malpighi, Hewson observed that neutral salts can retard or completely inhibit the clotting of the blood. He found that when the blood cells are allowed to settle in salted blood, the clear lymph can be decanted off and stored. On dilution with water, the stored lymph coagulates spontaneously. Hewson expressed the view that, prior to clotting, the fibrin is present as a solute in the plasma. Half a century later, Johannes Müller in Bonn (1832) was able to confirm this opinion. He filtered off the blood cells from frog's blood before coagulation occurred.

Berzelius, and later Nasse (1836), made thorough analyses of plasma in order to determine its content of fibrin or rather its precursor, which Virchow (1847) named fibrinogen. Later, this name was also adopted by Denis de Commercy (1859), who had until then used the expression "plasmine". He wrote: "Je crois devoir répéter encore que je n'entends pas dénommer ainsi la fibrine liquide, la fibrine plasmique, mais bien la matière nullement fibrineuse qui est l'origine de cette fibrine, la lymphe coagulable, la substance que j'appelais sérofibrine, et qu'on pourrait aussi désigner sous le nom de fibrinogène, si un pareil mot pouvait être admis. Elle est liquide dans le sang, et, dès qu'il est tiré de ses vaisseaux, elle ne tarde pas à se dénaturer."

Denis showed that fibrinogen can be salted out from plasma, kept incoagulable by means of sodium sulphate, on saturation with sodium chloride. Denis was also the first to suggest the possibility of precipitating globulins by dilution of the plasma with water. He observed that the fibrinogen precipitated with sodium chloride can be dissolved completely in water, but that some 10 minutes later it clots spontaneously, and then gradually redissolves. Denis' *Mémoire sur le sang* appeared in 1859, two years before Alexander Schmidt presented his coagulation theory.

In 1876–1880, Hammarsten published his studies on fibrinogen. He purified fibrinogen through repeated precipitations with saline at half saturation. He stated that pure fibrinogen solutions do not coagulate spontaneously, that the coagulability with thrombin more or less rapidly diminishes on storage, and that its solubility

is dependent on the presence of neutral salts. Moreover, exposure to a temperature of +52 to +55°C is sufficient to cause denaturation (Hammarsten 1876). Denaturation of plasma fibrinogen at +56 to +58°C was independently described by Frédéricq (1877).

Hammarsten's method formed the basis of the standard procedure for preparation of fibrinogen. Modifications of his method have been made by Rettger (1909), Howell (1910), Bordet (1920), Florkin (1930), Eagle (1935) and Chargaff (1943). Rettger increased the stability of the fibrinogen solutions by treating them with barium phosphate, which adsorbs prothrombin. Bordet (1920) used tricalcium phosphate, whose prothrombin-adsorbing capacity had been described by Bordet and Delange (1914). Bordet and co-workers had previously observed that prothrombin can be adsorbed not only to calcium phosphate, but also to calcium fluoride (Bordet & Gengou 1904) and barium sulphate (Bordet & Delange 1912). Florkin, who studied the solubility of fibrinogen in salt solutions, carried out all procedures at low temperature in order to minimize the risks of denaturation.

In 1909, Mellanby described a method of preparing fibrinogen in which he precipitated the plasma globulins by dilution of plasma with water, a principle which, as stated by Mellanby, had already been used by Alexander Schmidt.

The precipitability of fibrinogen in ammonium sulphate solutions had been studied by Hofmeister as long ago as at the end of the 19th century. McLean (1920), Smith, Warner & Brinkhous (1934) and Milstone (1941) precipitated the fibrinogen from plasma at 25 to 30 per cent saturation with ammonium sulphate. Before precipitation, Smith *et al.* treated the plasma with $Mg(OH)_2$ in order to remove the prothrombin. Milstone found that the fibrinogen preparations were usually contaminated by a lytic factor, which could, however, be removed by reprecipitating the fibrinogen with ammonium sulphate ten times.

Astrup & Darling (1942), who compared various methods of preparing fibrinogen, claimed that ammonium sulphate precipitation of the fibrinogen in plasma treated with tricalcium phosphate gave the best product with respect to both purity and stability.

Although neutral salts are usually employed for precipitation and purification of fibrinogen, buffer salts have also been used. Thus Florkin (1930) and Jacques (1943) utilized its low solubility in concentrated phosphate buffers.

Fibrinogen differs from most of the other plasma proteins by its low solubility at low temperatures. Freezing out the fibrinogen from plasma thus provides a simple way of obtaining a preparation of high purity (Ware, Guest & Seegers 1947). The plasma, frozen down to −30°C, is allowed to thaw gradually. The fibrinogen, which remains undissolved, is washed with chilled isotonic saline and then dissolved by increasing the temperature to 37°C.

Laki (1942) and Bagdy (1949) claimed that they had crystallized fibrinogen. The former gave, however, no data about the purity of the product, and Bagdy's crystalline preparation was coagulable only to 75 to 80 per cent. In a later publication by Szara & Bagdy (1953), describing a modified technique which should result in an increased purity of the preparation, no mention was made of any crystals.

At the end of the 1930's and the beginning of 1940's the Harvard group under the direction of Cohn carried out careful physicochemical investigations of the behaviour of proteins in ethanol–water solutions. During World War II, this resulted in the elaboration of the well-known 5-variable system for fractionation of plasma proteins. In Cohn's method 6 (1946) about 80 per cent of the fibrinogen is obtained

in fraction I, 40 to 50 per cent of which consists of fibrinogen. This fraction also contains small quantities of prothrombin and is therefore not stable in solution. Later improvements in the technique, such as collection of the blood over cation exchangers and in siliconized vessels, as well as adsorption of prothrombin on barium sulphate, to some degree increased the stability of fraction I (Surgenor, Alexander, Goldstein & Schmid 1951, Edsall & Dandliker 1951).

Further purification of fraction I by ethanol fractionation has been described by Morrison, Edsall & Miller (1948). With this procedure, fibrinogen is first precipitated at pH 5.1 at an ethanol concentration of 2 per cent, most of the contaminations remaining in solution. The fibrinogen fraction is then dissolved in a citrate buffer at pH 6.3, after which the globulins insoluble in the cold are removed at 0°C and an ethanol concentration of 0.5 to 1 per cent. The final product is described as containing between 93 and 98 per cent of protein coagulable with thrombin. However, on electrophoresis and ultracentrifugation, not even the purest preparations were found to be homogeneous. On ultracentrifugation, the main component had the S_{20} value of 8.5 S. A component with $S_{20} = 15$ S was also present, and sometimes a slower and a more rapidly sedimenting component as well.

The conditions under which fibrinogen precipitates are not the same for all species. Consequently, the aforementioned purification procedure, elaborated by Morrison, *et al.* for human blood, is not strictly applicable to bovine plasma. A modified procedure suitable for bovine plasma was therefore worked out by Morrison, Shulman & Blatt (1951), the final product, however, being coagulable to 90 per cent only.

In England, ethyl ether was used instead of ethanol for fractionation of human plasma proteins (Kekwick, Mackay & Record 1946, Kekwick & Mackay 1954). The fibrinogen thus obtained proved to be homogeneous on electrophoresis at pH 8 (Kekwick, Mackay, Nance & Record 1955). Ninety-seven per cent of the protein was coagulable with thrombin. It was claimed to be free from prothrombin and plasmin and, practically speaking, free from plasminogen or its proactivator. The authors made no distinction between the two last-mentioned components.

A new method for preparing fibrinogen

A prerequisite for the study of different clotting factors is access to pure fibrinogen and thrombin.

Stable fibrinogen preparations free from prothrombin and plasmin would be valuable also for clinical purposes.

In studying the mechanism of action of heparin and its co-factor on thrombin, we found the fibrinogen prepared by salt precipitation or by freezing out to be too impure and unstable to be used in the test system. Nor did alcohol fractionation, according to Morrison, Edsall & Miller (1948), give a completely satisfactory product regarding purity, although the stability of this fibrinogen was good.

In the following, a method is described for purifying the fibrinogen from Cohn's fraction I (method 6) by means of ethanol fractionation in the cold in the presence of glycine. The method is equally well applicable to human as to bovine plasma (Blombäck and Blombäck 1956).

Since proteins are, as a rule, more stable in the solid state, and dissolution of fibrinogen in the presence of prothrombin must involve undue risks, we tried to purify fraction I as far as possible by extracting the contaminants. Moreover,

extraction is more easily performed under sterile conditions than is fractional precipitation. The advantages of the extraction procedure have been pointed out by Cohn *et al.* (1950).

Dissociation of the protein complexes in fraction I with simultaneous quantitative separation of the components is, however, difficult to achieve by simple extraction with buffer solutions of varying pH and ionic strength. Preliminary experiments showed, however, that separation was highly effective when extraction was performed in the presence of glycine. Glycine in high concentration enhances the salting-out effect on the fibrinogen, i.e., reduces its solubility, whereas the salting-in effect on the contaminating proteins is large.

The salting out and salting in of amino acids, peptides and proteins in the presence of ions, dipolar ions and multipolar ions have been studied by various workers in this field. A mathematical analysis of the reactions involved has been presented by Debye and Hückel and by Kirkwood; and a survey of the investigations in this field has been given in a monograph by Cohn & Edsall (1943). The solubility of cystine and asparagine increases with the concentration of glycine (Cohn, McMeekin, Ferry & Blanchard 1939); this also applies, within certain limits, to the solubility of lactoglobulin and carboxyhaemoglobin as well as haemoglobin (Ferry, Cohn, Ondey & Blanchard 1939, Cohn *et al.* 1939, Richards 1937, Grönwall 1942). The increase in solubility can partly be ascribed to the increase in dielectric constant of the medium and to the dipole moments of the interacting molecules. Since for carboxyhaemoglobin the solubility is proportional to the dielectric constant only at low glycine concentration (below 0.2 *M*), it is supposed that specific effects also come into action (Richards). At high concentrations of glycine, a salting-out effect on the proteins appears which for carboxyhaemoglobin and haemoglobin is evidently above 1.5 molarity of the glycine.

Grönwall found that a multipolar substance like albumin also increases the solubility of the lactoglobulin and plasma euglobulin.

The effect of the low molecular dipoles on the solubility of cystine in water seems to be dependent on the number of non-polar groups in the molecule. Cohn *et al.* (1939) showed that alanine (one CH_2–group) salts-in cystine and asparagine less than does glycine, and that β-amino buturic acid, with two CH_2–groups, has an extremely slight salting-in effect, but a pronounced salting-out effect.

The results of these investigations found practical application in Cohn's method 10 (Cohn *et al.* 1950), in which the major part of the globulins is first precipitated and the different components are then separated by fractional extraction. Separation of the γ-globulins and the β_1-lipoproteins is facilitated by the presence of glycine.

Methods and material

pH determinations

Radiometer, Copenhagen, Type PHM 22 m. Determinations at room temperature.

Electrophoretic analyses

Fractionation was done in a Tiselius–Svensson apparatus. Use was made partly of a tris(hydroxymethyl)aminomethane buffer, pH 8.20 ± 0.05, ionic strength 0.2 (0.05 *M* Tris, 0.0014 *M* Na_2 EDTA (ethylenediaminotetraacetate), 0.15 *M* NaCl), conductance 10.44×10^{-3} ohm^{-1} cm^{-1} at 0°C; and partly of a phosphate buffer,

pH 6.65 ± 0.05 (0.026 *M* KH_2PO_4, 0.024 *M* K_2HPO_4, 0.0014 *M* Na_2 EDTA, 0.1 *M* NaCl), ionic strength 0.2, conductance 8.89×10^{-3} ohm $^{-1}$ cm^{-1} at 0°C.

The fibrinogen was dissolved in the buffer and dialyzed at +4°C for about 12 hours. The concentration of the protein was about 1 per cent. In the former analysis the temperature of the bath was kept throughout at + 1°C. In the latter, at pH 6.65 ± 0.05, it was found necessary to increase the temperature of the bath to +5°C, to avoid opalescence of the solution. The current used was generally 25 mA, but was adjusted according to the conductance of the buffer. Records were made during 20 to 24 hours.

Nitrogen determinations

Kjeldahl's micromethod after conbustion of the sample in sulphuric acid containing HgO + K_2SO_4 was used, 0.5 to 1.5 mg *N* in each analysis. During the investigations, the usual factor of 6.25 was used for deduction of the amount of protein including fibrinogen. Hammarsten (1880) found the deduction factors for fibrinogen and fibrin to be 6.02 and 5.91, respectively. Brand, Kassel and Saidel (1944) found the conversion factor for fibrin to be 5.92.

Fibrinogen determinations

Fibrinogen was determined as fibrin, after coagulation of the fibrinogen under standard conditions according to the syneresis method of Morrison (Morrison 1947, Ferry & Morrison 1947), although a higher concentration of thrombin was used (Saifer & Newhouse 1954). For the coagulation of fibrinogen we used a stock solution of thrombin containing 100 NIH units/ml, and kept frozen at −20°C. The thrombin was prepared according to Seegers *et al.* (1945) and contained between 60 and 80 NIH units per mg. No fibrinolytic activity was induced by this thrombin.

The optimal conditions for coagulation and syneresis are a fibrinogen concentration between 0.5 and 2 g/liter, a pH of about 6.35 and an ionic strength of 0.15 in the coagulation mixture (Ferry & Morrison 1947).

The coagulation procedure is briefly as follows: 1.0 ml of the sample to be determined, containing between 0.15 and 0.35 per cent of fibrinogen, is pipetted off into a small beaker (interior diameter 25 mm) and 2.0 ml of a phosphate buffer solution of pH 6.35 ± 0.05 and ionic strength 0.15 are added. This buffer contains 0.015 *M* phosphate in 0.075 *M* NaCl. 0.15 ml of the thrombin stock solution is added dropwise under mixing.

The clotting mixture is left standing at room temperature for two hours. The clot is then carefully poured on a small silk cloth (10 by 10 cm) with several underlying coarse filter papers and allowed to synerize completely. It is then immediately washed three times with 0.15 *M* NaCl, after which the synerized clot is wound up on a glass rod. In extracts and supernatants after precipitation, in which the fibrinogen concentration is low, 4.0 ml of the buffer were added to 5.0 ml of the sample and the mixture clotted with 0.45 ml of thrombin solution.

The clot can be analyzed for nitrogen according to micro-Kjeldahl. However, in most cases the protein content of the clot was determined by measuring the extinction at 282 mμ, after dissolving the clot in alkaline urea solution.

A similar method was devised by Jacobsson (1955) for determination of plasma fibrinogen. In Jacobsson's method, use is made of a slightly acid urea solution. This author recommended disodium EDTA as an anticoagulant. Jacobsson found

clots obtained on addition of thrombin to citrated plasma insoluble in urea solution. We have, however, found that these clots are readily soluble in different alkaline solutions of urea, such as urea buffered with Tris at pH 9, carbonate–bicarbonate buffer of pH 10–11, or sodium hydroxide urea solution.

We also tried to dissolve the clots in alkaline water solutions at +100°C and then determine the fibrinogen with the combined biuret and phenol reagent method of Lowry (1951). In our hands, however, this method did not show as good reproducibility as the urea method; moreover, it is desirable to make a standard curve each time.

The method we used may be described in brief as follows: The glass rod with the synerized clot is placed in a test tube with a ground-in stopper. Ten ml of 40 per cent urea in 0.2 *N* NaOH are added. After at least 60 minutes, when the clot is dissolved, 1 ml of 0.3 *M* NaCl is added and the contents are carefully mixed. The extinction values are read in a Beckman spectrophotometer, type DU, at 282 mμ in a 1 cm quartz cuvette, against alkaline urea containing the same amount of NaCl solution. Slit: about 0.3 mm. The extinction values should be read 2–4 hours after addition of the urea solution. The calculation of fibrinogen is described below.

The extinction read should be between 0.200 and 0.500, since these values correspond to the optimal fibrinogen concentration for coagulation and syneresis. The coagulability of fibrinogen is constant in this concentration range. At lower concentrations the yield of fibrin tends to decrease, and at fibrinogen concentrations below 0.02 per cent in the clotting mixture (extinctions below 0.100) the yield of fibrin is about 5 to 10 per cent too low. The standard deviation (S.D.) of a single observation, as calculated from 77 double determinations of clots within the aforementioned extinction limits, was found to be $\pm$ 0.004.

Ratio of coagulable protein to total protein in the fibrinogen preparations

This ratio is a measure of the purity of the fibrinogen. In glycine-free fibrinogen solutions, the nitrogen content of the fibrin and the total nitrogen are determined (micro-Kjeldahl). When the fibrinogen solutions contain traces of glycine, the fibrin and the total protein were determined spectrophotometrically after dissolution in the urea solution: 1.0 ml of fibrinogen solution (0.15 to 0.3 per cent fibrinogen, which was usually dissolved in 0.3 *M* NaCl) was pipetted off into a test tube, and 10 ml of 40 per cent urea in 0.2 *N* NaOH were added. The extinction was read at 282 mμ within two to four hours after the addition of urea. If the extinctions are read immediately or within 30 minutes the values will be some per cent too low, as there is a slight increase in the extinction during this time. Fibrin clots, even when formed from glycine-containing fibrinogen solutions, gave the same values (within 1.5 per cent) by the micro-Kjeldahl method as by the urea method, though the Kjeldahl method demands somewhat larger concentrations of fibrinogen than the optimal ones for clotting and syneresis.

The fibrinogen and protein contents are calculated in the following way:

$$\frac{E_{1\text{ cm}}^{282\text{ m}\mu} - b}{a} = X,$$

in which a denotes the slope of the standard curve, the value of which is obtained by simultaneous micro-Kjeldahl analyses of a glycine-free fibrinogen sample, prepared according to Seegers' method. b is the intercept on the ordinate of the standard curve, and X = mg of fibrinogen per ml original sample. We have found $a = 0.148$ and $b = 0.010$.

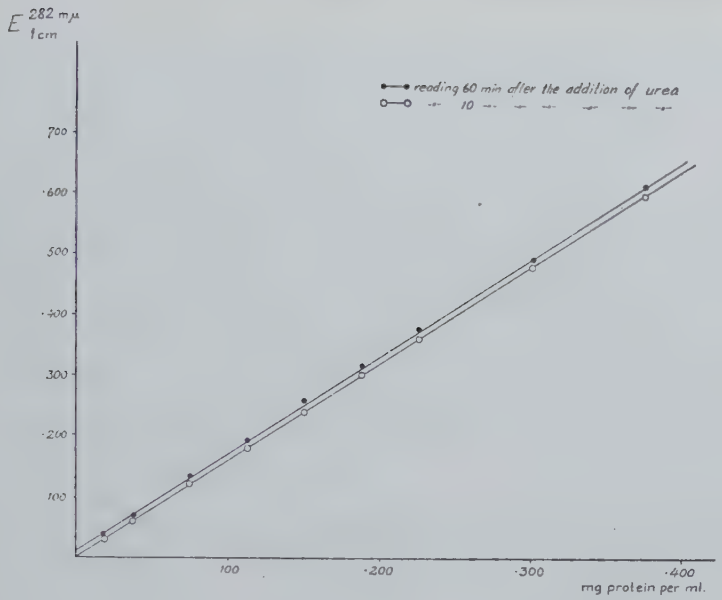


Fig. 1.

Lambert–Beer's law applies in extinctions between 0.030 and 1.00. If the extinctions are read within 10 minutes after the urea has been added, the curve will pass through the origin, but after 30 minutes it fits the equation $y = aX + b$, in which y denotes the extinction at 282 mμ (Fig. 1). This curve will remain constant for at least four hours. The standard deviation (S.D.) of a single value from the calculated curve, obtained from 137 values between the extinctions 0.03 and 0.6, was found to be $\pm$ 0.005.

The extinction varies considerably from one type of protein to another. $E\,{}^{1\ \text{per cent}}_{1\ \text{cm}}$ at 282 mμ was 16.17 for fibrinogen in alkaline urea solution, as against only 5.17 for human albumin. Consequently, the reliability of the urea method for determining the ratio of coagulable to total protein in fraction I depends on the similarity in the extinction coefficients of the different proteins in this fraction. Fortunately, the extinction coefficients of the proteins in fraction I appear to lie close to one another. This is apparent from Table 1A, which gives the coagulability of glycine-free fibrinogen preparations of varying purity, as determined by the micro-Kjeldahl and by the urea method. For comparison, the values for plasma have been included in the table. It is evident that as far as fibrinogen preparations with a coagulability of more than 90 per cent are concerned, both methods give practically the same results.

Table 1A.

Prep. no.	Coagulability		Difference in per cent
	Micro-Kjeldahl	Urea method	
Bovine plasma . .	7.3 (4)	12.5 (2)	41.6
F 35 B. M. . . .	66.5 (4)	68.7 (4)	3.2
F 38 B. M. . . .	75.9 (4)	77.0 (3)	1.4
F 36 B. M. . . .	92.8 (4)	92.9 (4)	0.1

Bracketed figures denote the number of double determinations performed on different days.

Occlusion tendency

A problem discussed by various authors is the occlusion of other proteins in the fibrin clot and, consequently, its importance for the ratio of coagulable to total protein. The occlusion of plasma protein in clots from partly purified fibrinogen appears to be of consequence only when the ratio of total protein to coagulable protein is high, about 10 or more (Morrison 1947, Ferry & Morrison 1947). Saifer & Newhouse found a high occlusion tendency in human citrate plasma. This does not, however, agree with Jacobsson's experience (1955) using EDTA plasma. He found the occlusion to be about 2 per cent. In our experiments, in which we added fibrinogen (80 per cent coagulable with thrombin) to human serum, we found an occlusion of protein in the clot amounting to a mean of 3.2 per cent (4 determinations) when the quotient of total protein to fibrinogen was between 10 and 20. If the quotient was below 10, we found no occlusion. This may indicate that the occlusion in the clots of highly purified human fibrinogen is very low.

The occlusion of thrombin in the fibrin clot seems to be negligible (Jacobsson 1955).

Determination of prothrombin

The prothrombin content was determined by the method of Owren & Aas (1951). This procedure determines the sum of the prothrombin and proconvertin.

Determination of plasminogen

The plasminogen was determined on heated plasminogen-free fibrin plates. The assay of plasminogen on such plates has been described by Astrup & Müllertz (1952) and by Lassen (1952).[1] It was found necessary to heat the plates for one hour at + 85°C to ensure complete inactivation of the plasminogen. Since bovine or human plasminogen cannot be activated directly, when streptokinase is used as an activator, but only via a proactivator present in human blood (Müllertz & Lassen 1953, Troll & Sherry 1955), the plasminogen must be determined in the presence of this proactivator. The method used by us was briefly as follows:

Reagents.—Proactivator: human plasma in 1 : 50 dilution with 0.15 *M* NaCl. Streptokinase: Varidase Lederle 5000 units/ml. Trypsin stock solution: 0.1 mg of trypsin (N × 6.25)/ml 0.005 *N* HCl, kept in the cold.

Procedure.—2.0 ml of the sample (buffered with Tris of pH 7.8) are activated with 0.25 ml of proactivator solution and 0.25 ml of streptokinase at room temperature for a few minutes. 0.03 ml of this solution is then added to the fibrin plate, and the latter is incubated at 37°C for 18 hours. The product of two perpendicular diameters, an expression for the digested area, is measured. In the blank, the buffer is substituted for the sample. If the plates are adequately heated, there is no digestion by the blank. The activity of the sample in several dilutions is read from the trypsin standard curve (0.02–0.4 γ trypsin).

Plasminogen was also determined with tosylarginine methyl ester as a substrate, according to Troll, Sherry & Wachman (1954), but this method was not, as a rule, sufficiently sensitive.

Determination of plasmin

Plasmin was determined according to Astrup & Müllertz 1952 on fibrin plates that had not been preheated. The procedure resembles that for determining plas-

[1] Plastic plates (150 × 150 mm) with a fibrin layer of 1.5 mm were used by us. The substrate was bovine fibrinogen (0.1 per cent) in Tris buffer pH 7.8, ionic strength 0.15.

minogen, except that streptokinase and the proactivator are not included. The plasmin content of the fibrinogen preparation can be roughly assessed by allowing the latter to stand in a test tube at 37°C and noting the time necessary for lysis.

Determination of the proactivator of plasminogen

Only human fractions were studied, as bovine plasma cannot be activated with streptokinase alone. The proactivator was activated with streptokinase and studied on unheated fibrin plates (prepared from 85 to 90 per cent coagulable fibrinogen) containing plasminogen in excess. The resulting plasmin activity was expressed in per cent of plasma (diluted 1 : 1000 to 1 : 6000 before determination).

Procedure.—2.0 ml of sample + 0.25 ml of streptokinase + 0.25 ml of Tris buffer, pH 7.8. Otherwise, the method was the same as that described for the determination of plasminogen. The reading of the digested area is facilitated by adding traces of phenol red indicator immediately before reading.

Determination of antihaemophilic globulin

The antihaemophilic activity was determined (1) according to Biggs' thromboplastin generation test (1955) and (2) by measuring the clot-promoting effect on recalcified citrated plasma from patients with type A haemophilia (Nilsson *et al.* 1956 in preparation).

Determination of the co-factor of heparin

The method for determination of the co-factor, as elaborated by us, is based on the formation of a strong antithrombin by the co-factor in combination with heparin. This method will be the subject of a later publication.

Centrifuge

Unless otherwise stated, International Refrigerated Centrifuge, Model SR-3, was used.

Extraction and precipitation vessels

Precipitation and extraction of large quantities were carried out in glass vessels equipped with a stirrer, a thermometer and an arrangement for the inflow of alcohol or buffer solution. Extraction was performed under sterile conditions in a 1.5 litre centrifuge cup of stainless steel, provided with a lid. In the centre of the lid was a hole, in which was inserted a cylinder of steel; its inner surface was lined with a 4 mm thick bearing of teflon. A glass stirrer was introduced through the hole and provided with a tubing of stainless steel, which slid against the inner surface of the teflon bearing. During centrifugation the lid with the mixer was replaced by an ordinary lid with a rubber gasket.

Buffer solutions for the fractionations

Stock buffer.—Citrate buffer of pH 6.0 ± 0.05, ionic strength 0.3, was prepared by adjustment of the pH of a 0.055 *M* sodium citrate solution with concentrated hydrochloric acid.

A buffer with a pH of 6.35 ± 0.05 was obtained by adjustment of the aforementioned stock solution with 1 *N* NaOH. The increase in ionic strength is small.

Glycine-containing buffer solutions for the extractions.—Glycine, Pharmacopea Suecica Ed. XI, was used. A glycine–ethanol–citrate buffer of pH 6.0 was prepared in the following way: 75 g glycine, 65 ml absolute ethanol and citrate buffer of pH 6.0 ± 0.05 to 1000 ml.

An extraction solution with a pH of 7.0 to 7.2 was obtained directly by adding to an 0.055 *M* trisodium citrate solution glycine to a concentration of 1 *M*, and ethanol to a concentration of 6.5 per cent.

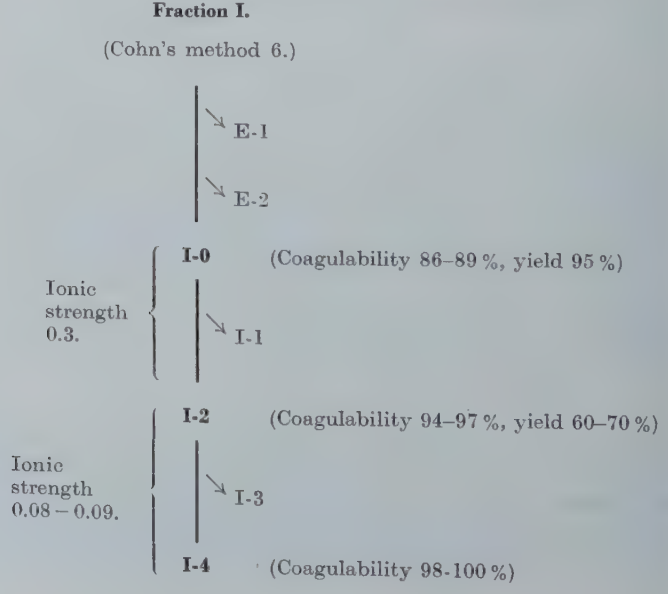
For sterile preparations, all solutions were prepared with sterile and pyrogen-free water, autoclaved and stored under sterile conditions.

Preparative procedure

Collection of the blood.—The blood was allowed to run into a solution of trisodium citrate (one part 3.8 per cent trisodium citrate, $Na_3C_6H_5O_7 \times 2H_2O$, to 9 parts of blood). The human blood was collected directly in ordinary, sterile blood-donor flasks, fitting the MSE centrifuge, model P. The blood cells were separated off by centrifugation at 1000 *g* (max.) and +10°C for 60 minutes. When the blood was prepared under unsterile conditions, it was centrifuged at 2000 *g* for about 50 minutes in centrifuge flasks.

Fraction I was precipitated by the method of Cohn *et al.* (1946) as soon as possible, always within 7 hours of collection of the blood, in order to minimize the loss of antihaemophilic globulin. After the addition of ethanol, the mixture was allowed to stand at −3°C under gentle stirring for one hour to make the precipitation more complete. The wet weight of the fraction was between 25 and 30 g per litre human plasma and 40 and 50 g per litre bovine plasma. This fraction contains between 40 and 50 per cent of protein coagulable with thrombin.

Scheme of Fractionation.



Extraction 1 (E-1).—For this purpose a citrate buffer, pH 6.0 ± 0.05, containing 6.5 per cent ethanol and 1 *M* glycine, ionic strength about 0.3, was used. 100 g wet weight of fraction I was extracted with 1000 ml of chilled buffer solution (−3°C), which was slowly added under cautious stirring until a homogeneous paste was obtained. The rest of the buffer was added and the suspension stirred for one hour at −3°C. The mixture was then centrifuged at 2000 *g* for 10–12 minutes. Temperature −3°C.

Extraction 2 (E-2).—This was performed under the same conditions as E-1. Bovine material was, however, extracted at pH 7.0–7.2. Human material can also be extracted at this pH but the yield is then a few per cent lower. Centrifugation was performed at 2000 *g* for 10 minutes; temperature −3°C. The precipitate was denoted as I-0. It can be stored at −20°C.

Fibrinogen containing AHG for intravenous use (human source)

Immediately after the extractions I-0 is dissolved in citrate buffer of pH 6.35 ± 0.05, the first portion of the buffer being chilled and gradually added under stirring. About 400 ml of citrate buffer are added for every 100 g of the original fraction I. The vessel is then transferred to a water bath with a temperature of +30°C, and the suspension stirred at this temperature until all material is dissolved, or for at most 30 minutes. The concentration of protein is usually between 1.5 and 2.0 per cent.

The solution is centrifuged at 2000 *g* at room temperature, in order to remove formed elements. The supernatant is then filtered through a layer of Hyflo Super Cel or through a Jena Glass filter G4, and immediately afterwards through a bacteriologic glass filter (Jena glass filter G5M, pore size 1.5–1.0 μ). The solution is then freeze-dried under sterile conditions.

The coagulability, i.e., the ratio of coagulable to total protein in fraction I-0, is 87–89 per cent. The yield is about 95 per cent of the fibrinogen in fraction I. However, loss of fibrinogen during filtration may be considerable, so that the final yield in the sterile preparations is not more than 70–80 per cent.

Before filtration, the yield of the antihaemophilic globulin in I-0 is almost 100 per cent of that in fraction I. Heavy losses were sometimes observed during the filtration process.

Further purification of fibrinogen

Precipitate I-0 is dissolved at +30°C in citrate buffer of pH 6.35 ± 0.05 to a protein concentration of about 1.5%. The solution is filtered, if it is cloudy, through an ordinary filter paper or centrifuged for 20 minutes and then diluted further with citrate buffer, pH 6.35 ± 0.05, and with 1 *M* glycine, dissolved in this buffer, until the protein concentration becomes 0.67 per cent and the glycine concentration 0.12 *M*. For every 100 g (wet weight) of fraction I the final volume of the solution will be about 1.3 litre.

Precipitation of I-1. (Our designation for the fraction, which contains the cold-insoluble globulins.)—To every 1000 ml of the dissolved fraction I-0, cooled to 0°C, a chilled mixture of 23.6 ml of absolute ethanol, 21.7 ml of 1 *M* glycine in citrate buffer pH 6.35 ± 0.05 and 135.7 ml of citrate buffer pH 6.35 ± 0.05, altogether 181 ml, is added in about 30–45 minutes. The protein concentration will then be 0.57 per cent, the ethanol concentration 2 per cent, the molarity of glycine 0.12 and the ionic strength about 0.3. The temperature is kept at 0°C throughout. The solution

is allowed to stand for 30 minutes at 0°C during gentle stirring. It is then centrifuged at 0°C at 2000 *g*. The precipitate, which is fairly small and mucous, consists of globulins insoluble in the cold and of fibrinogen. The coagulability with thrombin is about 70 per cent, and 15–20 per cent of the fibrinogen is lost in this precipitate.

Precipitation of I-2.—This is obtained by slowly adding to 1000 ml of the supernatant of I-1 375 ml of cold citrate buffer with a pH of 6.35 ± 0.05 containing 69.4 ml of absolute ethanol, so that the protein concentration will be about 0.35 per cent, the ethanol concentration 6.5 per cent, the glycine concentration 0.09 *M*, and the ionic strength about 0.3. During the addition, the temperature is lowered from 0°C to −4°C. The suspension is stirred gently for at least one hour. The fine white precipitate is centrifuged at 2000 *g* for 20 minutes at −4°C.

It is important that stirring be gentle so as to avoid the formation of foam during addition. If, however, foam does form, it will be thrown up to the surface on centrifugation and should then be removed. The sediment, which consists of purified fibrinogen, can either be dissolved in 0.3 *M* NaCl and stored frozen at −20°C, or dissolved in citrate buffer at pH 6.35 ± 0.05 and freeze-dried. The coagulability lies between 94 and 97 per cent and the yield between 65–75 per cent of the fibrinogen in fraction I.

Procedure and conditions for further purification of fraction I-2

For the further purification of I-2, bovine and human material had to be treated in different ways.

A. *Human material.*—Fraction I-2 is dissolved in citrate buffer of pH 6.35 ± 0.05, ionic strength 0.3, to a protein concentration of 0.7 per cent. Two parts of 0.75 *M* glycine solution are then added dropwise during stirring. After this dilution the pH is 6.4–6.5, the ionic strength 0.1 and the glycine concentration 0.5 *M*. The solution is then cooled to 0°C, whereby a small granular precipitate is sometimes obtained which, however, need not be removed before the precipitation of 1-3.

I-3.—This fraction is precipitated under the following conditions. Ethanol 0.75 per cent, glycine 0.5 *M*, protein concentration 0.21 per cent, pH about 6.5, and ionic strength 0.09. To every 1000 ml of the diluted fraction I-2 cooled to 0°C, 100 ml of a chilled 0.5 *M* glycine solution containing 8.25 ml of absolute ethanol are added.

The mixture is then allowed to stand at 0°C for at least 2 hours during very slow stirring, for complete precipitation of the fraction.

The mucous precipitate formed is centrifuged off (2000 *g*, 0°C, 10 min.). The supernatant must be clear.

I-4.—This fraction is precipitated in 6.5 per cent ethanol and a protein concentration of about 0.14 per cent. The glycine content is 0.45 *M*, pH about 6.5 and ionic strength 0.08.

To every litre of the supernatant, 123 ml of 53.3 per cent chilled ethanol are added dropwise under stirring. During this addition, the temperature is lowered from 0°C to −4°C. A finely granular white precipitate is formed. Centrifugation (2000 *g*, −4°C, 15 min.). The precipitate is then dissolved in 0.3 *M* NaCl or in citrate buffer of pH 6.35. The solution should be clear. The yield of fibrinogen from human fraction I-2 is 60–70 per cent. The ratio of coagulable to total protein is 98–100 per cent.

B. *Bovine material.*—Fraction I-2 is dissolved in 0.3 *M* NaCl to a protein concentration of about 1.8 per cent. To each 100 ml of this solution are added dropwise

480 ml of a mixture containing 145 ml of 2 *M* glycine, 55 ml of distilled water and 280 ml of a phosphate buffer of pH 7.3–7.5, ionic strength 0.1 (45 ml 0.2 *M* KH_2PO_4 + 155 ml 0.2 *M* K_2HPO_4 + distilled water to 1000 ml).

After this dilution, the pH is 7.0–7.1, the glycine concentration 0.5 *M* and the ionic strength considered to be 0.1. The solution is then cooled to 0°C.

I-3.—This fraction is precipitated under the following conditions. Ethanol 0.75 per cent, glycine 0.5 *M*, protein concentration 0.3 per cent, pH 7 and ionic strength about 0.09. To every 1000 ml of the diluted fraction I-2, cooled to 0°C, 100 ml of a chilled 0.5 *M* glycine solution containing 8.25 ml of absolute ethanol are added. The mixture is then allowed to stand at 0°C for at least 2 hours during gentle stirring. The coarse precipitate, which consists of cold-insoluble proteins and about 6–10 per cent of the fibrinogen in fraction I-2, is centrifuged off. The supernatant must be clear.

I-4.—This fraction is precipitated at 6.5 per cent ethanol and a protein concentration of about 0.15 per cent. The glycine content is 0.45 *M*, pH about 7 and the ionic strength about 0.08. To every 1000 ml of the supernatant 123 ml of 53.3 per cent ethanol are added under stirring. During this addition the temperature is lowered from 0°C to −4°C. A finely granular white precipitate is formed. Centrifugation (2000 g, −4°C, 15 min.). The precipitate is dissolved in 0.3 *M* NaCl or in citrate buffer pH 6.3–6.4. It can be stored frozen at −20°C for at least one year, or freeze-dried. The yield from fraction I-2 is 80–90 per cent. The ratio coagulable to total protein is 98–100 per cent before freeze-drying.

Experimental and results

The starting material

The starting material has been fraction I. The coagulability for five different bovine batches was 27.2, 45.2, 45.1, 41.3, 44.2 per cent and for four human batches 44.3, 53.9, 49.0, 45.5 per cent. The fibrinogen content in fraction I has been calculated by assuming fraction I = I-0 + E-1 + E-2 (see scheme of fractionation), a sum which agrees within 95–100 % (Table 1 B) with the value obtained by subtracting the fibrinogen in the supernatant of fraction I from the fibrinogen in plasma. The occlusion tendency in plasma is not taken into account.

Solubility of bovine fibrinogen in glycine–ethanol–citrate buffer mixtures

A study of the solubility of fibrinogen at various concentrations of glycine showed that, at constant ethanol concentration, pH and ionic strength, the solubility in-

Table 1 B. Calculation of the fibrinogen content of fraction I.

The figures refer to the amount of fibrinogen in grams recovered from one litre of plasma. H = human, B = bovine.

Fraction	Preparation				
	F36H	F44H	F46H	F47H	F62B
Plasma	2.487	2.695	2.584	2.528	7.195
Supernatant . .	−0.553	−0.323	−0.362	−0.579	−0.487
	1.934	2.372	2.222	1.949	6.708
I-0 + E-1 + E-2 .	1.876	2.358	2.219	2.005	6.750

creased with the concentration of glycine up to 0.3–0.5 molarity, the salting-in effect (Fig. 2). At higher concentrations the salting-out effect was predominant. It is remarkable that the salting-out effect appeared already at relatively low glycine concentrations.

Influence of the concentration of glycine and of ethanol, of the pH, ionic strength and temperature on the extraction of contaminants from fraction I[1]

Glycine and ethanol concentration.—On extraction of fraction I with 3 per cent ethanol, ionic strength 0.3 and different concentrations of glycine (Table 2), the ratio of coagulable to total protein in the precipitate increased with increasing concentration of the glycine (cf. Table 3). As is seen, the loss of fibrinogen in the supernatant increased up to 0.5 *M* glycine, and then decreased. These changes also agree with the solubility of the fibrinogen with increasing concentration of glycine, when use is made of purer fibrinogen preparations (Fig. 2).

An increase in the ethanol concentration to 6.5 per cent, at a glycine concentration of 1 *M*, did not appreciably decrease the solubility of the contaminants (cf. Tables

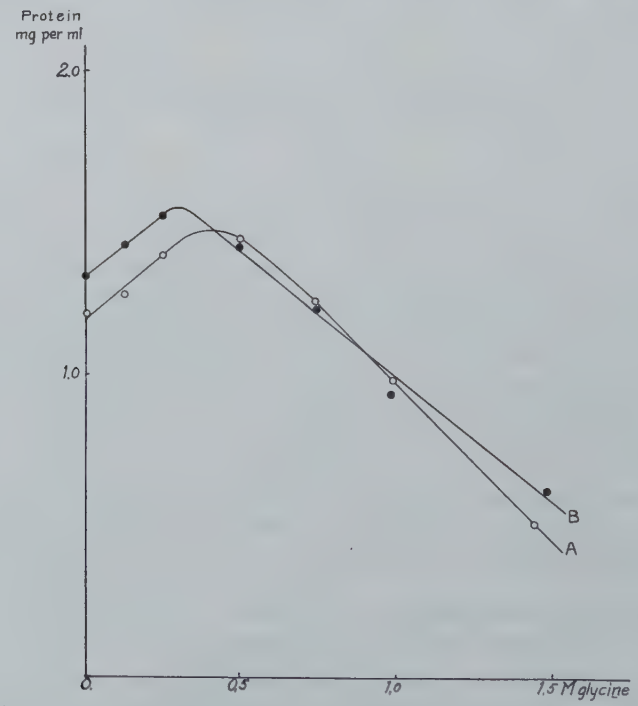


Fig. 2. The effect of glycine on the solubility of bovine fibrinogen.
Experimental: *A*. Fibrinogen (coagulability 90 per cent) in citrate buffer, pH 6.0, ionic strength 0.3, containing different amounts of glycine, was cooled to 0°C. Ethanol was gradually added under gentle stirring to a concentration of 2.0 per cent and the mixture was allowed to stand at 0°C for one hour. The protein concentration in the final mixture was 0.4 per cent. After centrifugation, the protein concentration in the supernatant was determined. *B*. Fibrinogen (coagulability 99 per cent). Ethanol was added to 3.0 per cent and the mixture was allowed to stand for three hours at 0°C. The other conditions were as described for exp. A.

[1] Unless otherwise stated, the experiments were carried out on bovine fraction I (coagulability 40–50 per cent).

Table 2. Influence of glycine on extraction of fraction I.
3 per cent ethanol, citrate buffer pH 6.0, ionic strength 0.3, temperature −2°C.

Glycine *M*	Coagulability per cent		Per cent yield in solid phase of total coagulable substance in fraction I
	Supernatant	Solid phase	
0	13.4	73.6	85
0.125	14.7	78.4	84
0.25	15.0	80.0	82
0.50	15.2	78.1	83
0.75	13.7	78.5	87
1.00	12.8	83.3	87
1.25	10.9	80.6	87

Table 3. Influence of glycine on extraction of fraction I.
6.5 per cent ethanol, citrate buffer pH 6.0, ionic strength 0.3, temperature −2°C.

Glycine *M*	Coagulability per cent		Per cent yield in solid phase of total coagulable substance in fraction I
	Supernatant	Solid phase	
0	3.8	60.6	98
0.25	5.9	74.9	97
0.50	6.8	77.7	96
1.00	—	81.5	95

Table 4. Influence of ethanol on extraction of fraction I in the absence of glycine.
Citrate buffer pH 6.0, ionic strength 0.3, temperature −2°C.

Ethanol per cent	Coagulability per cent		Per cent yield in solid phase of total coagulable substance in fraction I
	Supernatant	Solid phase	
0	38.9	79.5	55
3.0	13.4	73.6	85
6.5	3.8	60.6	98

2 and 3). The solubility of the fibrinogen was, on the contrary, considerably decreased and the yield of fibrinogen thus increased, for example, from 87 per cent with 3 per cent ethanol to 95 per cent with 6.5 per cent ethanol (at glycine concentration of 1 *M*).

Extraction in the absence of glycine, but with the same buffer and ethanol concentration, does not give such high dissociation of the fibrinogen complex in fraction I. Better results can be obtained with a buffer that does not contain glycine, if the ethanol concentration is reduced (Table 4). The same increase in purity as in the presence of glycine cannot, however, be obtained without a large loss of fibrinogen.

Influence of pH.—As can be inferred from Table 5, the purity of the fibrinogen in the solid phase increased somewhat on extraction with solutions having a higher pH.

Table 5. Influence of changes in pH of the extraction mixtures on coagulability and yield.

6.5 per cent ethanol, citrate buffer with varying pH containing 1 *M* glycine, ionic strength 0.3, temperature −2°C.

pH	Coagulability per cent		Per cent yield in solid phase of total coagulable substance in fraction I
	Supernatant	Solid phase	
6.00	7.8	82.0	95
7.05	7.8	83.0	95
7.25	7.0	85.2	96
7.85	8.3	85.1	95
8.60	10.4	85.6	93

The solubility of bovine fibrinogen was, on the whole, unchanged between pH 6.0 and 8.0, above which level it increased, with a resulting decrease in the yield. Morrison *et al.* (1951) found that the solubility of human fibrinogen increases more with the pH than does that of bovine fibrinogen. In the experiments recorded in Table 10, F10H–F13H were prepared with one extraction at pH 6.0 and subsequent extraction at pH 7.0. It is evident that the yield was lower than that from the corresponding bovine preparations. In the final method, we extracted the human fraction I twice at pH 6.0. Bovine fraction I is extracted the second time at a somewhat higher pH, preferably 7.0–7.2. After one extraction at pH 6.0, the coagulability of the solid phase was between 80 and 83 per cent; the second extraction resulted in an increase to 88 per cent. A third extraction did not appreciably improve the coagulability. The yield after two extractions, as calculated on fraction I, is 95 per cent for both human and bovine fibrinogen.

Ionic strength.—The solubility of fibrinogen in the absence of glycine is highly dependent on the ionic strength of the solution. In the presence of glycine in high concentration, with a resulting high dielectric constant of the medium, the solubility is inappreciably influenced by changes in ionic strength, as may be inferred from Table 6. In this experiment, fraction I was extracted with citrate buffer of varying ionic strength, in the presence of glycine.

An increase in the ionic strength from 0.1 to 0.3 had but little influence on the solubility of the fibrinogen. Cohn, McMeekin & Blanchard (1938) showed that in the presence of glycine in high concentration, changes in the ionic strength had only a slight effect on the solubility of cystine, a finding they ascribed to a decreased ionic amino acid interaction at high dielectric constant.

The ionic strength of the extractions in the final procedure was about 0.3.

Temperature.—A variation in the temperature at which extraction was carried out (between 0°C and −5°C) influenced neither the coagulability nor the yield of the final products (Table 7). In the final procedure, extraction was performed at −3°C to −4°C.

Table 6. Influence of ionic strength of the extraction solution on coagulability and yield.

6.5 per cent ethanol, citrate buffer pH 6.0, glycine 1 *M*, temperature −2°C.

Ionic strength	Coagulability per cent		Per cent yield in solid phase of total coagulable substance in fraction I
	Supernatant	Solid phase	
0.1	6.0	77.2	97
0.15	6.3	81.1	97
0.2	6.4	82.0	96
0.3	—	81.6	95

Table 7. Influence of temperature on coagulability and yield during extraction of fraction I.

6.5 per cent ethanol, citrate buffer pH 6.0, glycine 1 *M*, ionic strength 0.3.

Temperature °C	Coagulability per cent solid phase	Per cent yield in solid phase of total coagulable substance in fraction I
0	81.5	95
−2	81.6	95
−5	81.6	96

Table 8. Influence of the concentration of ethanol on precipitation of I-1, loss of fibrinogen and coagulability of the final product.

As starting material we used a bovine fraction I stored at −20°C. The precipitation was performed in citrate buffer pH 6.35 ± 0.05, ionic strength 0.3 at a glycine concentration of 0.12 *M* and a protein concentration of 0.57 per cent.

Ethanol per cent	Loss of fibrinogen in per cent of fibrinogen in I-0	Coagulability per cent	
		Fraction I-1	Fraction I-2
0.5	3.8	74.2	90.0
1.0	4.8	72.0	91.1
1.5	13.0	76.0	92.8
2.0	21.4	84.0	93.9

Conditions for precipitation of cold-insoluble globulins denoted as fraction I-1

Morrison, Edsall & Miller (1948) precipitated this globulin fraction at 1 per cent ethanol in a citrate buffer of pH 6.35 and ionic strength of 0.3 at 0°C. We studied the influence of different ethanol concentrations on the loss of fibrinogen in precipitating fraction I-1, as well as on the purity of the final product (Tables 8 and 9). As is apparent from the tables, an ethanol concentration of 0.5–1.0 per cent is not sufficient to obtain a final product of good coagulability. The loss of fibrinogen with these concentrations was, however, small.

In order to ensure removal of most of the cold-insoluble globulins, we precipitated fraction I-1 at 2 per cent ethanol, glycine 0.12 *M*, in a citrate buffer of pH 6.35 ± 0.05, ionic strength 0.3, and at a protein concentration of 0.57 per cent, under which conditions the solubility of the fibrinogen was relatively high.

Table 9.

Preparation (H = human, B = bovine)	Ethanol per cent	Loss of fibrinogen in per cent of fibrinogen in I-0	Coagulability per cent	
			Fraction I-1	Fraction I-2
F54B	1.25	9.8	78.1	93.1
F55B	1.75	14.9	65.8	95.1
F10H	2.0	22.9	72.3	95.1
F11H	2.0	16.9	73.0	96.2
F12H	2.0	24.1	71.9	96.0

A protein concentration of about 0.6 per cent was taken as the highest possible for precipitation of fraction I-1. A higher concentration is hardly to be recommended, since the loss of fibrinogen would then be too large, owing to its relatively low solubility at low temperatures. Higher glycine concentration has no advantage, and between 0.2 and 0.35 *M* incomplete precipitation of fraction I-1 is noted.

Fraction I-1 is a coarse blue-white precipitate; it dissolves readily at room temperature but flocculates on lowering the temperature to +4°C. After coagulation of the fibrinogen in fraction I-1 with thrombin, a cold-insoluble component is obtained in the supernatant. As pointed out by Morrison, Edsall & Miller (1948) and Edsall *et al.* (1955), its solubility is much lower than that of fibrinogen.

Analysis of the conditions under which purified fibrinogen (fraction I-2) was precipitated from the supernatant of fraction I-1

Fraction I-2 was precipitated at an ethanol concentration of 6.5 per cent, in citrate buffer pH 6.35 ± 0.05, ionic strength 0.3, at 0.09 *M* glycine and at a protein concentration of about 0.35 per cent. A higher concentration of glycine decreases the yield at the concentration of ethanol in question. After precipitation of the cold-insoluble globulins, Morrison *et al.* precipitated the purified fibrinogen with 8 per cent ethanol and an ionic strength of 0.15. Since ethanol in a concentration of 6.5 per cent and an ionic strength of 0.3 gives just as large a yield, we preferred to use these conditions, since this precipitation also implies a considerable purification of the fibrinogen. Whereas the coagulability of the supernatant after precipitation of fraction I-1 was only 90–92 per cent, the purified fibrinogen, fraction I-2, had a coagulability of 94–97 per cent. It consisted of a finely granulated, white precipitate readily soluble at room temperature in 0.3 *M* NaCl or in citrate buffer, pH 6.35 ± 0.05 and ionic strength 0.3. Table 10 gives a survey of some fibrinogen preparations (human and bovine) with the degree of purity and the yield of the different fractions. It is evident that, with this purification process, it is possible to recover about 60 to 70 per cent of the fibrinogen in Cohn's fraction I with a coagulability of 94 to 97 per cent.

Table 10.

Preparation (H = human, B = bovine)	Fraction				
	I-0		I-1	I-2	
	Coagulability per cent	Yield from fraction I per cent	Coagulability per cent	Coagulability per cent	Yield from fraction I per cent
F10H*	87.2	85.9	72.3	95.1	61.0
F11H*	88.7	88.0	73.0	96.2	72.3
F12H*	84.7	92.2	71.9	96.0	65.0
F13H*	89.3	87.4	—	—	—
F36H	84.5	95.5	—	94.2	67.5
F44H	88.3	97.3	71.8	94.8	68.5
F46H	86.4	98.1	64.6	93.7	75.1
F55B	88.7	99.0	65.9	95.6	68.1
F49B	86.0	90.5	78.0	96.5	69.0
F57B	87.8	93.5	79.8	96.9	65.5
F52–6B	89.0	93.3	76.0	94.7	80.0
F62B	91.7	94.1	88.6	95.8	55.6

* Here, the second extraction of the human fraction I was performed at pH 7.0–7.2, for which reason the yield in fraction I-0 was somewhat lower than for the other human preparations.

Refractionation and further purification of fraction I-2

Refractionation under the conditions described above, in an attempt to remove any residual cold-insoluble proteins or other contaminating proteins from the purified fibrinogen fraction, resulted in no further increase in the purity of the fibrinogen. Nor did variations in the glycine content (the glycine concentration was varied between 0 and 0.5 *M*) produce any demonstrable improvement.

As mentioned earlier, Cohn *et al.* (1938) pointed out that the effect of dipolar ions in solution is influenced by other ions present, and that the effect decreases with

Table 11.

Preparation (H = human, B = bovine)	Fraction				
	I-2 Coagulability per cent	I-3		I-4	
		Yield from fr. I-2 per cent	Coagulability per cent	Yield from fr. I-2 per cent	Coagulability per cent
F60B	93.7	—	—	85.5	99.2
F61B	93.4	14.0	96.9	78.5	99.3
F62B	95.8	5.6	87.4	88.6	99.5
F44H	94.8	31.9	94.2	56.9	98.4
F46H	93.7	22.2	82.7	69.2	98.1
F47Hb	91.9	27.3	87.5	68.4	98.9
F47Hd	91.9	34.4	86.5	59.0	98.5

rising concentration of the latter. Since the earlier purification steps were carried out at relatively high ionic strength (0.3), it was considered of interest to investigate whether fractionation at lower ionic strength (below 0.1) in the presence of glycine would enhance the purity of the product from fraction I-2. It then proved possible, with a low ethanol concentration, to remove a further cold-insoluble protein complex (I-3). Removal of this complex raised the coagulability of the subsequent fibrinogen fraction (I-4) to 98–100 per cent. Table 11 gives a survey of the degree of purity and the yield of the different fractions of the final purification procedure.

Influence of pH, ethanol and protein concentration on the precipitation of I-3 with respect to purity and yield of the final fibrinogen fraction I-4

Human fraction I-2 of 93–96 per cent coagulability was used as the starting material. The experiments described below were usually carried out in citrate buffer and at a glycine concentration of 0.5 *M*.[1] A stock solution of fraction I-2 in 0.3 *M* NaCl was diluted with glycine and a buffer of ionic strength 0.1. About 20 to 40 per cent of the mixture consisted of buffer solution, the final ionic strength before precipitation of I-3 being 0.1. After removing fraction I-3, precipitated at an ionic strength of 0.09 at 0°C, the fraction I-4 was precipitated, when not otherwise stated, by increasing the ethanol concentration to 6.0–6.5 per cent, at an ionic strength of about 0.08, a protein concentration of about 0.15–0.20 per cent and a temperature of −3°C. In the supernatant of this fraction about 5 per cent of the total protein in fraction I-2 was found, but practically no fibrinogen could be discovered.

Influence of pH.—As is apparent from Table 12, the loss of fibrinogen in fraction I-3 increased markedly with decreasing pH. To obtain the higher pH (7.1 and 7.7), use was made of a phosphate buffer. The maximum coagulability of the fibrinogen

Table 12. Influence of pH on precipitation of fractions I-3 and I-4.

Initial fraction I-2: coagulability 93 per cent.
I–3: 1.5 per cent ethanol, protein concentration 0.40 per cent.
Other conditions: see text.

pH	I-3			I-4		
	Per cent yield		Coagulability per cent	Per cent yield		Coagulability per cent
	Protein	Fibrinogen		Protein	Fibrinogen	
5.8	68.0	69.5	94.1	29.1	30.4	96
6.0	60.6	56.4	86.5	32.7	34.4	98.2
6.35	53.5	52.6	88.1	41.3	45.4	97.7
7.1 *	43.8	43.8	90.8	50.4	53.7	97
7.7 *	22.9	22.2	87.7	69.1	73.5	96.7

* Phosphate buffer.

[1] Preliminary attempts to make the fractionation at low ionic strength in the absence of glycine were not successful. Furthermore, the yield was about 25 per cent lower. Lower glycine concentrations, i.e. 0.3 *M*, resulted in incomplete precipitation of fraction I-3. The final fibrinogen solution was opalescent and had lower coagulability.

in I-4 was obtained after precipitation of I-3 between pH 6.0 and 6.4. This suggested that these cold-insoluble globulins do not differ from those precipitated at high ionic strength (0.3) (Edsall 1955), as far as the pH dependency of precipitation is concerned.

In the final procedure, human fraction I-3 was precipitated at pH 6.3–6.4.

In bovine material, optimal precipitation of I-3 was obtained also at pH 7.0, at which pH the yield of fibrinogen in fraction I-4 was considerably higher (80–90 per cent of I-2); consequently, this pH was used in the final procedure.

Ethanol concentration.—Optimal precipitation of the cold-insoluble protein complex under the conditions otherwise described above occurred at an ethanol concentration of 0.75 per cent (Table 13).

Protein concentration.—Although the low solubility of fibrinogen has long been known, the concentration of protein has seldom been considered in its purification. As can be inferred from Table 14, too high a protein concentration when precipitating

Table 13. Influence of different ethanol concentrations on precipitation of fraction I-3.

Original fraction I–2: 93 per cent coagulability.
I–3: pH 6.35 ± 0.05, protein concentration 0.40 per cent.
Equilibration for 1 hour.
I–4: 6 per cent ethanol, pH 6.95.
Other conditions: see text.

Ethanol per cent	I-3			I-4		
	Per cent yield		Coagulability per cent	Per cent yield		Coagulability per cent
	Protein	Fibrinogen		Protein	Fibrinogen	
0.42	48.8	46.9	87.0	47.3	49.9	95.7
0.75	56.3	56.2	88.2	35.9	39.8	98.7
1.5	65.4	67.0	94.5	28.3	30.4	98.7

Table 14. Influence of protein concentration on fractionation of I-2.

Original fraction I–2: 93–96 per cent coagulability.
I–3: pH 6.35 ± 0.05, ethanol concentration 1.5 per cent.
I–4: 6 per cent ethanol. pH 6.35.
Other conditions: see text.

Species	Protein concentration: I-2 per cent	I-3			I-4		
		Per cent yield		Coagulability per cent	Per cent yield		Coagulability per cent
		Protein	Fibrinogen		Protein	Fibrinogen	
Human	0.28	37.4	36.3	94.9	62.6	63.8	99.5
Human	0.33	43.6	43.3	96.1	52.7	54.3	98.4
Human	0.33	43.1	43.6	93.9	51.9	54.8	98.2
Bovine	0.33	48.0	47.2	93.3	47.8	50.3	99.8
Bovine	0.35–0.36	52.0	51.1	91.2	44.9	46.6	98.3
Human	0.35–0.36	52.5	49.5	87.4	41.3	45.4	97.7
Human	0.56	58.0	57.4	88.1	38.8	41.5	95.1

I-3 was associated not only with a large loss, but also with a decrease in the purity of the fibrinogen in fraction I-4. This is probably to be ascribed to a decreased dissociation of the protein complexes at a high fibrinogen concentration. We considered 0.2–0.3 per cent to be a suitable concentration for precipitation of I-3.

Occurrence of clotting factors in the various fractions

Fraction I consists of fibrinogen and other less readily soluble plasma proteins. Almost all the antihaemophilic activity is also found in this fraction. The protein coagulable with thrombin usually represents 40 to 50 per cent of the fraction. In addition, the fraction contains varying amounts of most of the other plasma proteins, probably co-precipitated by occlusion. Cohn *et al.* (1946) and Morrison, Edsall & Miller (1948) found them to be 7 per cent albumin, 8 per cent alpha globulins, 15 per cent beta globulins and 9 per cent gamma globulins. In addition to the so-called antihaemophilic globulin, this fraction contains small amounts of most of the factors participating in coagulation.

The prothrombin is of particular interest, its quantitative removal being necessary for the stability of the fibrinogen.

It is evident from Table 15 that the prothrombin content of human fraction I represents 2 per cent of that of plasma. It was calculated by addition of the content found in the two extracts E-1 and E-2 and in the precipitate I-0. Approximately 95 per cent of the prothrombin in fraction I was removed by the two extractions. The purified fibrinogen (fraction I-2) contained no ascertainable prothrombin activity. In corresponding bovine fractions, it was not possible to ascertain prothrombin activity even in fraction I-0, probably because of the low prothrombin content of bovine plasma, which is only 15–20 per cent of that of human plasma. The small quantity of residual prothrombin in fraction I-0 from human plasma does not appear to be sufficient to impair the stability of the fibrinogen from the practical viewpoint. Under sterile conditions, this fraction proved stable in solution for several weeks.

The antihaemophilic globulin (AHG) activity was present almost quantitatively in fraction I-0 (80–100 per cent of that of fraction I). However, on further purification of the fibrinogen, the activity disappeared completely.

Table 15. Prothrombin activity in the various fractions.

Fraction	Prothrombin per cent		Per cent of the prothrombin present in fraction I*		Relative activity per mg protein	
	F11H	F12H	F11H	F12H	F11H	F12H
Plasma	100	100	—	—	184	152
E-1 (1st extract)	1.84	1.63	80.5	86.0	49	43
E-2 (2nd extract)	0.32	0.18	14.0	9.5	24	32
I-0	0.126	0.085	5.5	4.5	2.0	1.0
I-2	< 0.0088	< 0.0096	< 0.3	< 0.5	< 0.1	< 0.1

* Calculated from the total content of prothrombin present in E-1, E-2, and I-0.

The final purified fibrinogen possessed no antihaemophilic activity. Fraction I-0 had a high AHG content and was stable in solution; consequently, this fraction was considered to be most suitable for clinical purposes. An investigation of the occurrence of AHG in the different fractions will be the subject of a separate paper (Blombäck, B., Blombäck, M. and Nilsson, I. M., in preparation).

Plasmin, the active fibrinolytic enzyme impairs the stability of fibrinogen, as does prothrombin. No plasmin activity could, however, be demonstrated in the fibrinogen fractions after extraction.

Plasminogen and the proactivator of plasminogen. As mentioned above, the proactivator was studied only in human fractions after activation with streptokinase.

Fraction I-2 still contained about 20 per cent of the content of the two enzymes in fraction I. Although the finally purified fibrinogen (fraction I-4) contained about 10 per cent of the plasminogen and the proactivator in fraction I, the tendency of these enzymes to separate from fibrinogen during the purification process was apparent as is seen in Tables 16A and B, where the total activity and the activity per mg protein are given. The plasminogen and the proactivator seem to follow each other during the purification procedure. From Table 16A it can be seen that the plasminogen content in bovine fractions is higher than in human.

The error of the methods used seems to be somewhat large (10–20 per cent).

The heparin co-factor—the factor in plasma which, together with heparin, forms a strong, rapidly acting antithrombin—occurs mainly in Cohn's fraction IV–1 (Koller & Derouaux). We found this fraction to contain 40 to 60 per cent of the co-factor activity of the plasma. Fraction I contained only an inappreciable amount, which could be removed quantitatively by the aforementioned extraction process.

Table 16 A. Plasminogen.

Plasminogen expressed in γ trypsin (N × 6.25). The total amount is calculated per litre plasma.

Fraction	F51H		F36H		F31H		F60B	
	Total γ trypsin	γ trypsin/ mg prot.	Total γ trypsin	γ trypsin/ mg prot.	Total γ trypsin	γ trypsin/ mg prot.	Total γ trypsin	γ trypsin/ mg prot.
Plasma . . .	1 660	0.028	2 255	0.038				
Supernat. fr. I	1 210	0.021	1 848	0.032				
E-1	137	0.102	214	0.180				
E-2	93	0.674	95	0.546				
I-0	288	*0.105*	68	*0.033*				
I-1	71	0.082						
Supernat. fr. I-2	68	0.800						
I-2	123	*0.074*	42	*0.034*	70	*0.057*	257	*0.136*
I-3	33	0.082	11	0.022	25	0.120	35	
Supernat. fr. I-4	115	7.500			60	0.590		
I-4	69	*0.054*	6	*0.011*	16	*0.021*	127	*0.084*

Table 16 B. Proactivator.

Relative content and activity of the proactivator of plasminogen in different fractions.

	Total activity in per cent of plasma	Rel. activity per mg protein
F12H		
Plasma	100	1.69
E-1	2.5	2.53
E-2	1.1	9.30
I-0	*2.1*	*1.13*
I-1	0.5	0.83
I-2 (supernatant)	0.6	3.60
I-2 (precipitate)	*1.3*	*1.09*
F31H, F51H		
I-2	*1.24, 1.69*	*0.94, 1.09*
I-4	*0.42, 0.63*	*0.50, 0.63*

Beha viour of the purified fibrinogen in free electrophoreses

On electrophoresis at pH 6.6, fraction I-2 (coagulability 96 per cent) migrated as an essentially homogeneous protein even in 20-hour runs (Fig. 3). The coagulability was unchanged during electrophoresis.

At pH 8.2 in Tris buffer, the purest fibrinogen (98–100 per cent) also migrated as a homogeneous protein during a 10-hour run. However, after 15 hours a slower migrating peak and after 19 hours a faster migrating peak appeared. Both of them are small, as seen in Fig. 4. After electrophoresis, the coagulability had decreased to 91.7 per cent, which might explain the appearance of the two smaller peaks.

Discussion

Fibrinogen is an important substrate in the study of many questions relevant to the coagulation of blood. It is known that it is difficult to obtain pure stable fibrinogen by salting out with electrolytes alone, the difficulty being due to co-precipitation of other substances together with the fibrinogen. Seegers' method of freezing-out the plasma fibrinogen represented a noteworthy advance. Using this method, we prepared fibrinogen with 90 to 94 per cent coagulability. These fibrinogen preparations, however, proved too labile for use for example in the study of the enzymatic conversion of fibrinogen to fibrin, or the inhibition of this process by heparin and its co-factor. Moreover, the yield was fairly poor.

Attempts to extract impurities from Cohn's fraction I with salt solutions and phosphate buffers also failed to give satisfactory results. These preparations nearly always contained active plasmin, with the result that fibrinogen solutions containing 0.2–0.3 per cent protein were completely digested after some hours at room temperature. By adsorption we succeeded in removing the plasmin from the fibrinogen and in obtaining stable preparations. However, the fibrinogen obtained by this technique was very impure, and the yield was low (10–20 per cent).

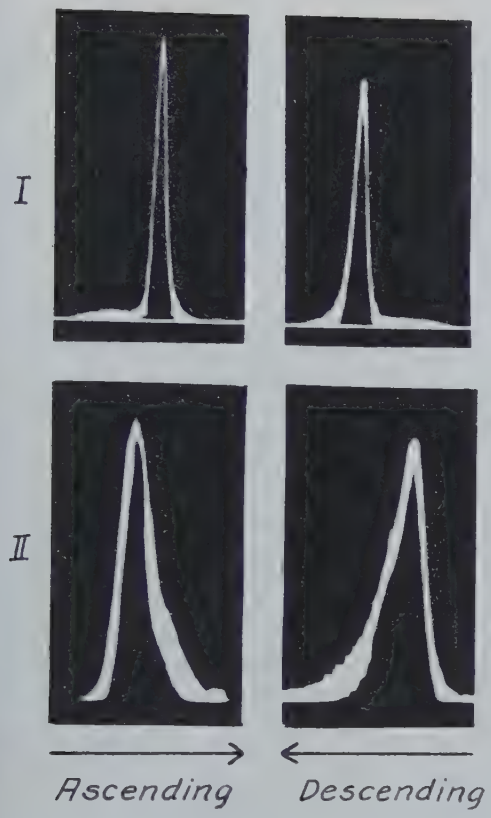


Fig. 3.

Fig. 3. Electrophoresis in phosphate buffer, pH 6.65, ionic strength 0.2. Protein concentration at start 1 per cent. Exposures: I, after 5 hours, slit 2; and II, after 20 hours, slit 6.

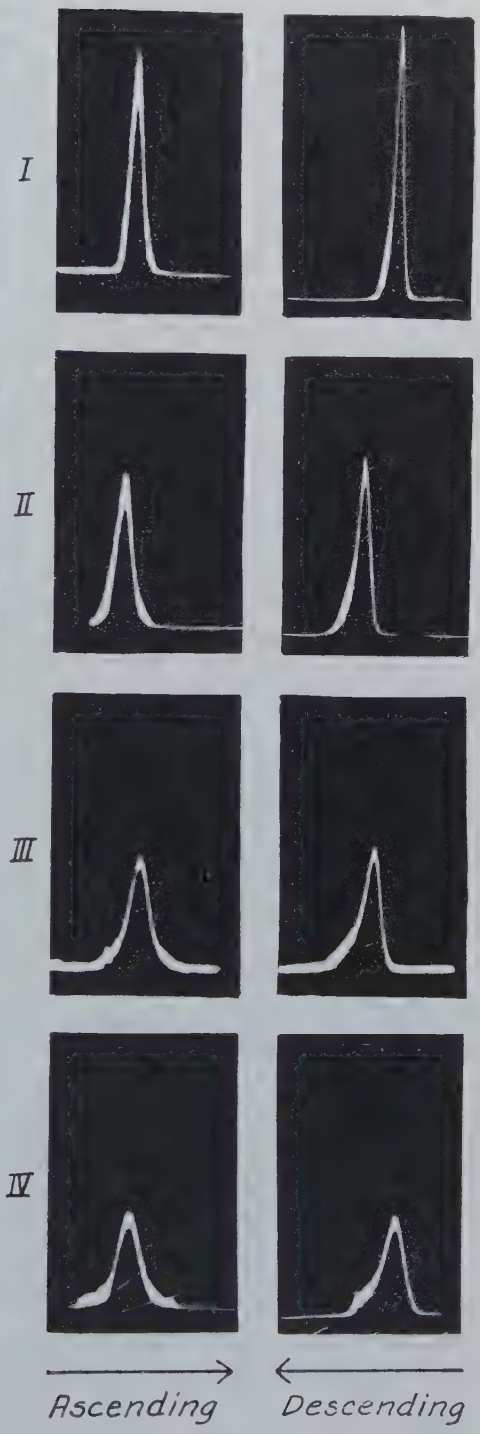


Fig. 4.

Fig. 4. Electrophoresis in Tris buffer, pH 8.20, ionic strength 0.2. Protein concentration at start 1 per cent. Exposures: I, after 6 hours; II after 9 hours; III, after 15 hours; IV, after 20 hours.

The human fibrinogen obtained by the method of Morrison, Edsall & Miller (1948) was satisfactory as far as stability is concerned. The coagulability, as determined by the method described in the present paper, was, however, never more than 92 per cent, as against the 93–98 per cent claimed by the authors. The difference is partly to be ascribed to the fact that Morrison *et al.* determined the fibrin with the gravimetric method which, according to Kekwick *et al.* (1955), may give values 2 per cent too high. When bovine plasma was used as starting material, however, the purification process did not give such good results. The coagulability was, as a rule, between 65 and 80 per cent. The antihaemophilic globulin was destroyed by the preparative procedure.

A matter of considerable importance in the method of Morrison *et al.* is the removal of the cold-insoluble globulin fraction, a component occurring in fraction I and first observed by them. Since fraction I had been obtained from a large number of donors, they discussed the possibility of the cold-insoluble globulins being derived from donors with pathologic cryoglobulins similar to those described by Waldenström (1944), Abrams, Cohen & Meyer (1949) and Lerner, Barnum & Watson (1947), among others. However, we consistently found cold-insoluble proteins in fraction I prepared from plasma from one or a small number of donors. We also found that a cold-insoluble globulin fraction could be separated off from bovine fraction I. This argues definitely for its being a normal component of plasma. Like Morrison *et al.*, we precipitated it at pH 6.35 and an ionic strength of 0.3, but we used a somewhat higher alcohol concentration and a small amount of glycine in order to increase the tendency to separation. The similarity between the cold-insoluble globulin fraction separated by Morrison *et al.* and that described by us is evident. The fraction, making up 15–20 per cent of the protein in fraction I, has the consistence of a gel; it is readily soluble at +30°C, but precipitates when chilled to +4°C. In human material it consists to about two thirds of fibrinogen and to one third of non-coagulable globulins. In bovine material the percentage of fibrinogen is somewhat higher.

The coagulability of fraction I-1, the first separated cold-insoluble globulin fraction, is lower than that of the second cold-insoluble globulin fraction (I-3). A discussion of the implications of this difference is scarcely feasible, since the accuracy of the determination of the ratio of coagulable protein to total protein in these fractions seems to be somewhat unreliable. This is due to the lability of the fractions when separated from the rest of the fibrinogen. We have often observed spontaneous flocculation of them even at room temperature. Reasons were put forward by Edsall *et al.* (1955) indicating that the cold-insoluble proteins form a complex together with fibrinogen. Morrison, Edsall & Miller found a considerable variation in the concentration of the non-clotting component in different batches of plasma, namely, between 0.15 and 0.45 g/litre plasma. In four batches of human plasma, we found the corresponding component to range from 0.11 to 0.16 g/litre of plasma (average 0.14 g/litre). In five batches of bovine plasma, we found a somewhat wider variation, i.e., between 0.11 and 0.33 g/litre (average 0.18 g/litre).

Fibrinogen prepared according to Kekwick *et al.* (1955) by ether fractionation is claimed to be to 97 per cent coagulable with thrombin. A freeze-dried preparation[1] showed, with the method used by us for determination of fibrinogen, a coagulability of 95.5 per cent, as compared to the stated 97 per cent before freeze-drying. This can be regarded as good agreement if it is borne in mind that, according to Kekwick

[1] Kindly supplied by Dr. R. A. Kekwick, The Lister Institute, London.

et al., freeze-drying decreases the coagulability by about 1 per cent. This fibrinogen is claimed to be free from plasminogen or its proactivator, or from both. The test used by these authors does not permit any differentiation between the two factors, since both of them are necessary for the demonstration of lysis.

In the procedure used by us, plasminogen is determined in the presence of an excess of proactivator on a plasminogen-free substrate, and the proactivator is determined in the presence of an excess of plasminogen. With these methods, it is possible to demonstrate fibrinolytic activity as low as that corresponding to 0.02 micrograms of trypsin. Even our purest human preparations contained traces of plasminogen and its proactivator. The separation tendency during the purification procedure is, however, apparent and it is possible that further refractionations at low ionic strength could remove these enzymes completely.

Judging by the coagulability and electrophoresis, the fibrinogen prepared by us is at least as pure as that prepared by Kekwick *et al.* by ether fractionation. The yield of fibrinogen in the procedure described here is considerably higher than in the procedure of Kekwick.

Although antihaemophilic globulin was recovered quantitatively in Cohn's fraction I, its use has been markedly limited by the instability of this fraction. Further purification of fraction I by earlier methods involved a loss of activity (Kekwick & Mackay 1954). Purification of fraction I by treatment with glycine, using the method described here, is not associated with any appreciable loss of activity. The antihaemophilic globulin can be recovered in the partly purified fibrinogen fraction I-0. Its antihaemophilic activity calculated per mg protein is 30–50 times that of the plasma.

Summary

A method for the preparation of human and bovine fibrinogen is described. The starting material (Cohn's fraction I) was extracted with glycine–citrate–ethanol mixtures. The extractions were performed at a glycine concentration where the fibrinogen was nearly insoluble, but the contaminating proteins went into solution.

In this way, prothrombin and plasmin, which otherwise endanger the stability of the fibrinogen, were removed and a fibrinogen with a coagulability of 88 per cent, containing practically all the antihaemophilic globulin of fraction I, was obtained. Further purification of this fibrinogen by removing the cold-insoluble globulins at high ionic strength gives a fibrinogen with a coagulability of 95 per cent, and by removing another contaminating fraction of low solubility at low ionic strength a fibrinogen with a coagulability of 98–100 per cent is obtained.

The yield of the fibrinogen with 95 per cent coagulability was 60–70 per cent of the fibrinogen in fraction I. The fibrinogen was easily soluble and stable both in a lyophilized condition and in sodium chloride solution.

This investigation was carried out at the Chemistry Department II, Karolinska Institutet, Stockholm. We express our sincerest thanks to the head of the department, Professor Erik Jorpes, under whose guidance our work was done.

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REFERENCES

Abrams, A., Cohen, P. P., and Meyer, O. O., J. Biol. Chem. *181*, 237 (1949).
Astrup, T. and Darling, S., Acta Physiol. Scand. *4*, 45 (1942).
Astrup, T. and Müllertz, S., Arch. Biochem. Biophys. *40*, 346 (1952).
Bagdy, D., Hung. Acta Physiol. *2*, 18 (1949).
Biggs, R., Eveling, J., and Richards, G., Brit. J. Hemat. *1*, 20 (1955).
Blombäck, B. and Blombäck, M., Acta Chem. Scand. *10*, 147 (1956).
Bordet, J. and Gengou, O., Ann. Inst. Pasteur *18*, 26 (1904).
Bordet, J. and Delange, L., Ann. Inst. Pasteur *26*, 737 (1912).
—— Berlin. Klin. Wschr. *51*, 497 (1914).
Bordet, J., Compt. rend. Soc. Biol., Paris, *83*, 576 (1920).
Brand, E., Kassel, B., and Saidel, L. J., J. Clin. Invest. *23*, 437 (1944).
Chargaff, E. and Bendich, A., J. Biol. Chem. *149*, 93 (1943).
Cohn, E. J., McMeekin, T. L., and Blanchard, M. H., J. Gen. Physiol. *21*, 651 (1938).
Cohn, E. J., McMeekin, T. L., Ferry, J. D., and Blanchard, M. H., J. Physical Chem. *43*, 169 (1939).
Cohn, E. J. and Edsall, J. T., Proteins, Amino Acids and Peptides as Ions and Dipolar Ions. Reinhold Publ. Corp. 1943.
Cohn, E. J., Strong, L. E., Hughes, W. L. Jr., Mulford, D. J., Ashworth, J. N., Melin, M., and Taylor, H. L., J. Amer. Chem. Soc. *68*, 459 (1946).
Cohn, E. J., Gurd, F. R. N., Surgenor, D. M., Barnes, B. A., Brown, R. K., Derouaux, G., Gillespie, J. M., Kahnt, F. W., Lever, W. F., Liu, C. H., Mittelman, D., Mouton, R. F., Schmid, K., and Uroma, E., J. Amer. Chem. Soc. *72*, 465 (1950).
Denis, P.-S., Mémoire sur le sang. J. B. Baillière et fils, Paris 1859.
Eagle, H., J. Gen. Physiol. *18*, 531 (1935).
Edsall, J. T. and Dandliker, W. B., First Proc. Univ. Lab. Physical Chem. Relat. to Med. and Publ. Health, Harvard University. Reprinted Dec. 1951, p. 114.
Edsall, J. T., Gilbert, G. A., and Scheraga, H. A., J. Amer. Chem. Soc. *77*, 157 (1955).
Ferry, J. D., Cohn, E. J., Oncley, J. L., and Blanchard, M. H., J. Biol. Chem. *128*, Sci. Proc. xxviii (1939).
Ferry, J. D. and Morrison, P. R., J. Am. Chem. Soc. *69*, 388 (1947).
Florkin, M., J. Biol. Chem. *87*, 629 (1930).
Fredericq, L., De l'existence dans le plasma sanguin d'une substance albuminoïde se coagulant à +56°C. Gand 1877. (Extrait des Annales de la Société de Médecine de Gand.)
Grönwall, A., Compt. rend. trav. lab. Carlsberg. Sér. Chim. *24*, 185–232, Nos. 8–11 (1941–1944).
Hammarsten, O., Uppsala Läkarefören. förh. XI, 538 (1875–1876).
—— Pflügers Arch. Physiol. *19*, 563 (1879).
—— Pflügers Arch. Physiol. *22*, 431 (1880).
Hewson, W., The Works of William Hewson by George Gulliver. Sydenham Society, London 1846.
Howell, W. H., Am. J. Physiol. *26*, 453 (1910).
Jacobsson, K., Scand. J. Clin. Lab. Invest. *7*, Suppl. 14 (1955).
Jaques, L. B., Biochem. J. *37*, 344 (1943).
Kekwick, R. A., Mackay, M. E., and Record, B. R., Nature, London, *157*, 629 (1946).
Kekwick, R. A. and Mackay, M. E., Med. Research Council Spec. Report Series No. 286. London, Her Majesty's Stationery Office, 1954.
Kekwick, R. A., Mackay, M. E., Nance, M. H., and Record, B. R., Biochem. J. *60*, 671 (1955).
Laki, K., Z. Physiol. Chem. *273*, 95 (1942).
Lassen, M., Acta Physiol. Scand. *27*, 371 (1952).
Lerner, A. B., Barnum, C. P. and Watson, C. J., Amer. J. Med. Sci., *214*, 416 (1947).
Lerner, A. B., Watson, C. J., Ibid., 214, 410 (1947).
Lowry, O. H., Rosebrough N. J., Farr A. L., and Randall, R. J., J. Biol. Chem. *193*, 265 (1951).
McLean, J., Bull. Johns Hopkins Hosp. *31*, 453 (1920).
Malpighi, M., Opera omnia. London 1686.

MELLANBY, J., J. Physiol. *38*, 28 (1909).
MILSTONE, H., J. Immunol. *42*, 109 (1941).
MORRISON, P. R., J. Amer. Chem. Soc. *69*, 2723 (1947).
MORRISON, P. R., EDSALL, J. T., and MILLER, S. G., J. Amer. Chem. Soc. *70*, 3103 (1948).
MORRISON, P. R., SHULMAN, S., and BLATT, W. F., Proc. Soc. Exp. Biol. Med. *78*, 653 (1951).
MÜLLER, J., Poggendorffs Annalen, Berlin, *25*, 513 (1832).
MÜLLERTZ, S. and LASSEN, M., Proc. Soc. Exp. Biol. Med. *82*, 264 (1953).
NASSE, H., Das Blut. Bonn 1836.
OWREN, P. A. and AAS, K., Scand. J. Clin. Lab. Invest. *3*, 201 (1951).
RETTGER, L. J., Amer. J. Physiol. *24*, 406 (1909).
RICHARDS, M. M., J. Biol. Chem. *122*, 727 (1937).
SAIFER, A. and NEWHOUSE, A., J. Biol. Chem. *208*, 159 (1954).
SCHMIDT, A., Zur Blutlehre. Leipzig 1892.
—— Weitere Beiträge zur Blutlehre. Wiesbaden 1895.
SEEGERS, W. H., LOOMIS, E. C., and VANDENBELT, J. M., Arch. Biochem. *6*, 85 (1945).
SMITH, H. P., WARNER, E. D., and BRINKHOUS, K. M., Amer. J. Physiol. *107*, 63 (1934).
SURGENOR, D. M., ALEXANDER, B., GOLDSTEIN, R., and SCHMID, K., J. Physical & Coll. Chem. *55*, 94 (1951).
SURGENOR, D. M., First Proc. Univ. Lab. Physical Chem. Relat. to Med. and Publ. Health, Harvard University. Reprinted Dec. 1951, p. 72.
SURGENOR, D. M., ALEXANDER, B., GOLDSTEIN, R., and SCHMID, K., First Proc. Univ. Lab. Physical Chem. Relat. to Med. and Publ. Health, Harvard University. Reprinted Dec. 1951, p. 78.
SZARA, ST. and BAGDY, D., Acta Physiol. Acad. Sci. Hung. *4*, 229 (1953).
TROLL, W., SHERRY, S., and WACHMAN, J., J. Biol. Chem. *208*, 85 (1954).
TROLL, W. and SHERRY, S., J. Biol. Chem. *213*, 881 (1955).
VIRCHOW, R., Arch. Pathol. Anat. Physiol., Berlin, *1*, 547 (1847).
WALDENSTRÖM, J., Acta Med. Scand. *117*, 216 (1944).
WARE, A. G., GUEST, M. M., and SEEGERS, W. H., Arch. Biochem. *13*, 231 (1947).

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On the thermodynamics of ion exchange equilibria

II. Relative magnitude of formal osmotic free energy and formal ionic interaction free energy

By Lennart W. Holm

With 1 figure in the text

Introduction

In part I of this series of papers [1], a general thermodynamical equation was derived for the equilibrium of two ions between an ion exchanger and a surrounding solution by treating it as a Gibbs-Donnan equilibrium (eqn. (9) [1]). It was also shown that several formally different, although of course thermodynamically equivalent, equations were obtained by choosing different reference states for the activity coefficients. The distribution of the two ions at equilibrium is expressed by the selectivity coefficient:

$$K_m = \left(\frac{m'_A}{m''_A}\right)^{z_B} \cdot \left(\frac{m''_B}{m'_B}\right)^{z_A} \tag{1}$$

where m'_A and m''_A are the molalities of the ion A in phase ′ and ″, m'_B and m''_B the corresponding quantities of the ion B, and z_A and z_B the valences of A and B. From the equations mentioned above, it was obvious that the selectivity coefficient varies with the pressures on the two phases and with the compositions of the phases, since it contains the activity coefficients and the partial molal volumes of the exchanging ions. The main aim of this paper is to discuss this dependence in some detail.

Pressure dependence of the selectivity coefficient

In the study of the pressure dependence of the equilibrium distribution, it is only of interest to consider those cases when the compositions of the reference states are chosen constant and equal in the two phases, i.e. $(m'_A, m'_B)_{r_A} = (m''_A, m''_B)_{r_A} = (\underline{m}_A, \underline{m}_B)_{r_A}$ for the ion A and analogously for B. The symbols used are the same as those in part I. Furthermore we put the pressure on the surrounding solution (= phase ′) constant and equal to the atmospheric pressure, $p' = 1$.

If the pressures in all reference states are chosen constant and equal to one atmosphere, i.e. $p'_{r_A} = p''_{r_A} = p'_{r_B} = p''_{r_B} = 1$ we have for pressure-independent activity coefficiens according to part I (eqn. (18 a)):

$$RT \ln K_m = RT \ln \frac{\{\gamma'_B [1, (\underline{m}_A, \underline{m}_B)_{r_B}] (1, m'_A, m'_B)\}^{z_A}}{\{\gamma'_A [1, (\underline{m}_A, \underline{m}_B)_{r_A}] (1, m'_A, m'_B)\}^{z_B}} -$$

$$- RT \ln \frac{\{\gamma''_B [1, (\underline{m}_A, \underline{m}_B)_{r_B}] (1, m''_A, m''_B)\}^{z_A}}{\{\gamma_A [1, (\underline{m}_A, \underline{m}_B)_{r_B}] (1, m''_A, m''_B)\}^{z_B}} +$$

$$+ \int_1^{p''} \{z_B v''_A (p'', m''_A, m''_B) - z_A v_B (p'', m''_A, m''_B)\} dp. \qquad (2)$$

If we, on the other hand, choose the pressures in the reference states in the external solution phase constant and equal to one atmosphere as before, i.e. $p'_{r_A} = p'_{r_B} = 1$, but in the ion exchanger phase equal to the actual pressure in that phase, i.e. $p''_{r_A} = p''_{r_B} = p''$, which is regularly not constant, the following equation holds according to part I (eqn. (22 a)):

$$RT \ln K_m = RT \ln \frac{\{\gamma'_B [1, (\underline{m}_A, \underline{m}_B)_{r_B}] (1, m'_A, m'_B)\}^{z_A}}{\{\gamma'_A [1, (\underline{m}_A, \underline{m}_B)_{r_A}] (1, m'_A, m'_B)\}^{z_B}} -$$

$$- RT \ln \frac{\{\gamma''_B [p'', (\underline{m}_A, \underline{m}_B)_{r_B}] (p'', \underline{m}''_A, m''_B)\}^{z_A}}{\{\gamma''_A [p'', (\underline{m}_A, \underline{m}_B)_{r_A}] (p'', m''_A, m''_B)\}^{z_B}} +$$

$$+ \int_1^{p''} \{z_B v^0_A [p, (\underline{m}_A, \underline{m}_B)_{r_A}] - z_A v^0_B [p, (\underline{m}_A, \underline{m}_B)_{r_B}]\} dp. \qquad (3)$$

To simplify the formulas somewhat we will only consider univalent ions and choose infinitely dilute solutions as the reference states in both solution and ion exchanger phases. The choice of an infinitely dilute, aqueous solution as reference state even for the ion exchanger phase is commonly used and quite consistent when regarding the ion exchanger as a concentrated electrolyte solution.

In the following we will cease indicating the compositions of the reference states. Furthermore the pressure in the reference state will be indicated only when it is not constant; thus no indication of reference pressure means that it is constant and equal to 1 atmosphere. Similarly an activity coefficient is defined at one atmosphere if nothing else is obvious from the notation. Eqn. (2) can then be written:

$$RT \ln K_m = RT \ln \frac{\gamma'_B (m'_A, m'_B)}{\gamma'_A (m'_A, m'_B)} - RT \ln \frac{\gamma''_B (m''_A, m''_B)}{\gamma''_A (m''_A, m''_B)} +$$

$$+ \int_1^{p''} \{v''_A (p'', m''_A, m''_B) - v''_B (p'', m''_A, m''_B)\} dp \qquad (2\,a)$$

and eqn. (3):

$$RT \ln K_m = RT \ln \frac{\gamma'_B(m'_A, m'_B)}{\gamma'_A(m'_A, m'_B)} - RT \ln \frac{\gamma''_B[p'](p'', m''_A, m''_B)}{\gamma''_A[p''](p'', m''_A, m''_B)} + \int_1^{p''} \{v^0_A[p] - v^0_B[p]\}\, dp. \quad (3\,a)$$

Since the activity coefficients in the external solution, γ', are independent on the ion exchanger system, they are without fundamental interest in this connection and can thus be removed from the discussion. Both Gregor [2] and Glueckauf [3] tacitly assume the ions to be incompressible. Boyd and Soldano [4] make the same approximation but point out that although permitted in many cases it is sometimes an extremely poor approximation, especially at high pressures and with large differences between the compressibilities of the ions. In some cases, due allowance for the compressibilities may be made by means of published data [5]. Considering the accuracy obtainable for the activity coefficients in the ion exchanger phase however, one may assume v_A and v_B to be approximately constant. On doing so, and noting that the pressure on the ion exchanger phase, p'', is equal to the sum of the swelling pressure, π, of the ion exchanger and the atmospheric pressure, i.e. $p'' - 1 = \pi$, eqn. (2 a) reduces to:

$$RT \ln K_m = RT \ln \frac{\gamma'_B(m'_A, m'_B)}{\gamma'_A(m'_A, m'_B)} - RT \ln \frac{\gamma''_B(m''_A, m''_B)}{\gamma''_A(m''_A, m''_B)} + \pi\{v_A(m''_A, m''_B) - v_B(m''_A, m''_B)\}. \quad (2\,b)^1$$

Analogously, eqn. (3 a) becomes:

$$RT \ln K_m = RT \ln \frac{\gamma'_B(m'_A, m'_B)}{\gamma'_A(m'_A, m'_B)} - RT \ln \frac{\gamma''_B[p'](p'', m''_A, m''_B)}{\gamma''_A[p''](p'', m''_A, m''_B)} + \pi(v^0_A - v^0_B). \quad (3\,b)$$

The last two terms of these equations are different. In eqn. (2 b) the activity coefficients in the ion exchanger phase are defined exactly as is usual for water solutions, i.e. they are valid at 1 atmosphere and approach the value one when $\sum_i m_i \to 0$ *at a pressure of 1 atmosphere*. Consequently, they compensate only for part of the deviation from ideality because of ionic interaction, while the partial ionic volumes take care of the rest. These activity coefficients have, however, the advantage of being independent of the degree of crosslinking of the ion exchanger.

In eqn. (3 b) on the other hand, the activity coefficients in the ion exchanger phase are defined at the actual pressure on the resin phase, p'', and they approach the value one when $\sum_i m_i \to 0$ *at the actual pressure* p''; i.e. not when $p'' \to 1$ as was stated by Boyd and Soldano [4]. It may be mentioned, however, that their statement is true for the activity coefficients of eqn. (17) of part I, provided that the pressures in the reference states have been chosen equal to 1 atmosphere. The activity coefficients of eqn. (3 b) account completely for the ionic interaction, the partial ionic volumes being those at infinite dilution.

[1] An analogous formula has been derived by Glueckauf [3] in a somewhat different way.

Boyd and Soldano [4] introduced the names "ionic interaction free energy" and "osmotic free energy" for the last two terms of eqn. (3 b) respectively. These names are, however, not quite adequate since the osmotic free energy is represented not completely by the last term but also partially by the pressure dependence of the activity coefficients. For reasons already given, such a complete separation is likewise not feasible in eqn. (2 b). Nevertheless, the separation is made possible by regarding the volume difference $\{v_A(m_A'', m_B'') - v_B(m_A'', m_B'')\}$ in the last term of eqn. (2 b) as approximately equal to $(v_A^0 - v_B^0)$. When applying eqn. (2 b) in studies of cation and anion exchange equilibria, Soldano and co-workers [6, 7] made this approximation assuming the difference between the partial molal volumes of two ions at the actual conditions to be about equal to the same difference at infinite dilution. They justify this approximation by the fact that the contribution of the pressure-volume term is small in most cases (cf. below), and thus the error introduced is of second order importance.

The relative magnitudes of the last two terms of eqn. (3 b) have been discussed by Boyd *et al.* [4]. They consider the cation exchanger Dowex 50 (divinylbenzene (DVB) content 8 %) saturated with hydrogen and sodium ions in the proportion 1:1 and surrounded by a solution dilute enough to make the activity coefficient ratio there equal to unity. The essential points of their arguments are as follows.

1. Using published values for the partial molal volumes [5], they found that the pressure-volume term (π is here 137 atmospheres; the calculation of π will be described in part III) contributes only slightly more than 2 per cent to the experimentally measured value of log K_m. It may be mentioned that when considering the pressure dependence of the ionic volumes—neglected by Boyd *et al.* despite the fact that they refer the pair H^+–Na^+ to those combinations where this approximation is "extremely poor"—the contribution of the pressure-volume term decreases by 50 per cent.

2. The importance of the pressure-volume term is increased or better, the contribution of the activity coefficient term is decreased, when, like Gregor [2], we apply the Bjerrum-Harned-Stokes-Robinson theory [8 a] and choose the concentration unit in such a way that the volumes become the partial molal volumes of the hydrated ions. Using the same values for the volumes of the hydrated ions as Gregor, Boyd *et al.* found the contribution of the pressure-volume term to be 50 per cent of log K_m. To make a better estimate of the difference between the hydrated volumes, Boyd *et al.* extrapolated to zero crosslinking the measurements made by Pepper, Reichenberg, and Hale [9] on the difference between the external volumes of the hydrogen- and sodium-saturated ion exchangers. Thus they found the contribution of the pressure-volume term to be about 80 per cent. From this they concluded that the pressure-volume term is of major inportance when using hydrated ionic volumes even if the activity coefficient term is necessary to obtain a quantitative agreement with the measured value of the selectivity coefficient. This fact does not, however, entitle to complete neglection of the activity coefficients in the ion exchanger phase as suggested by Gregor [2].

Later, Reichenberg [10] has argued that the difference of the hydrated ionic volumes is equal to twice the difference of the external ion exchanger volumes. Accepting his arguments (for details, see ref. [10]) and the new value of the volume difference given by him, the pressure-volume term is found to amount to ~ 120 per cent of the value of log K_m. Thus, the above-mentioned conclusion by Boyd *et al.* remains, even though the reason is somewhat changed.

3. As an additional argument against the assumption that the pressure-volume term exclusively determines the equilibrium, Boyd *et al.* state: Since the swelling pressure is always greater than zero (at least in water solutions) and the volume difference is a constant (positive or negative) the pressure-volume term cannot account for any selectivity reversals, maxima (as found for instance for the equilibrium Na^+–H^+ [11]), or minima of the selectivity coefficient as a function of the ionic composition of the exchange resin. For the Na^+–H^+ case, the volume measurements by Pepper, Reichenberg, and Hale [9] have shown the swelling pressure to vary linearly with the composition of the ion exchanger, and consequently the same should be true for the value of log K_m supposing that it is dependent on the pressure-volume term alone.

A comparison similar to that of Boyd and Soldano has recently been carried out by Reichenberg and McCauley [12] for the K^+–Na^+ equilibrium with the same fundamental result.

Glueckauf [3] has argued that Gregor's use of the volumes of the hydrated ions at infinite dilution might not be acceptable at the high concentrations present in ion exchange resins. The criticism is formally not quite adequate, since the convention adopted in eqn. (3 b) (which was used by Gregor) postulates (hydrated) ionic volumes at infinite dilution, which volumes are, of course, independent of concentration. As a matter of principle, the choice of hydrated ionic volumes is however rather unfortunate because of the high concentrations in the ion exchanger, which result in a lack of water for the complete hydration of the ions present. The most important disadvantage is, nevertheless, the difficulty of obtaining significant values for the hydrated volumes.

The dependence of the hydration number on the method used for its determination is obvious from Bockris' [13] review of different methods. The results of Glueckauf and Kitt's [14, 15] isopiestic investigations of the hydration of cation exchangers also show that there is no reason for assigning a definite hydration number to a cation.

From measurements of ion exchange rates and the sorption of non-electrolytes in ion exchange resins, Pepper and Reichenberg [16] consider they can calculate an upper limit of 4–5 for the hydration number of the hydrogen ion. Their volume measurements [9] mentioned above give a difference between the hydration numbers of the hydrogen ion and the sodium ion equal to 5 [10]; consequently the lower limit of the hydration number for H^+ is 5, i.e. equal to the upper limit. These results seem, however, to be completely incompatible with the difference of 4 between the hydration numbers of the sodium and potassium ions, obtained by Reichenberg and McCauley [12] from volume measurements. A comparison with the values—H^+ 3.9, Na^+ 1.5, i.e. a difference of 2.4—obtained by Glueckauf and Kitt [15] from thermodynamical data assuming a certain sorption mechanism gives a further example of the difficulty of even finding a suitable convention in connection with the hydration of ions.

The selectivity coefficient in relation to the activity coefficient ratio in water solutions

Gregor and Bregman [17] have, especially in one paper pointed out several phenomena as arguments for the pressure-volume term being regarded as exclusively or at least predominantly determining the value of the selectivity coef-

ficient. It seems, however, possible to explain all these phenomena by considering the behaviour of the activity coefficients. Especially one of these arguments will be discussed here in detail.

Instead of showing the inablility of the pressure-volume term to explain the variation of the selectivity coefficient, as Boyd and Soldano [4] (cf. above) did, Gregor and Bregman try to show that the activity coefficient term is not equal to the selectivity coefficient. Since they did not know any actual values for the activity coefficients in the ion exchanger phase, they compared the selectivity coefficient with the ratio of certain activity coefficients in ordinary water solutions assuming the latter to represent the corresponding ratio in the ion exchanger. From the poor agreement obtained, they concluded that the activity coefficient term cannot account for the selectivity coefficient while the pressure-volume term should be of the utmost importance. This inference seems to be too hasty in view of the fact that, at least, as probable a reason for the deviation is the use of activity coefficients which may not be suitable substitutes for those actually applying to the ion exchanger.

As an example we will consider Gregor and Bregman's discussion of the selectivity coefficient, K_m, of the equilibrium between potassium and lithium ions (at $K^+ : Li^+ \sim 1$) in a PSS ion exchanger. They plotted K_m as a function of the concentration in the ion exchanger phase (corresponds to different degrees of crosslinking) and compared the curve obtained with the curve for the ratio of the mean molal activity coefficients of lithium chloride and potassium chloride in pure water solutions versus concentration. When doing this, they found a substantial deviation of the activity coefficient ratio from the value of the selectivity coefficient (cf. Fig. 5 of ref. [17]) and concluded that the activity coefficient ratio " bears no relationship to experimental values " of K_m. However, on checking one finds that the activity coefficient ratio given by Gregor and Bregman is considerably higher than the ratio which can be calculated from data given in the literature [8 b, 18]; furthermore the curve for $\gamma_{LiCl}/\gamma_{KCl} = fn(m)$ extends up to $m = 8$ although potassium chloride is known to be saturated even at $m = 4.7$. The diagram has consequently been redrawn here, see Fig. 1.

The ratio of the activity coefficients of lithium and potassium paratoluenesulfonates [18], $\gamma_{LiTol}/\gamma_{KTol}$, has also been included in the diagram. From a comparison of this ratio with the selectivity coefficient of the K^+–Li^+ exchange equilibrium, Bregman [19] concluded that the activity coefficient ratio "agrees surprisingly well" with values of K_m at low degrees of crosslinking. This conclusion is quite the contrary to the one which Bregman arrived at earlier in collaboration with Gregor [17]. As pointed out by Bregman [19], it is unfortunate that the activity coefficients of the paratoluenesulfonates are not known at high concentrations; the solubility imposes, however, a limit also in this case. The only possible conclusion from Fig. 1 is that the ratio $\gamma_{LiTol}/\gamma_{KTol}$ agrees perhaps slightly better with K_m than the ratio $\gamma_{LiCl}/\gamma_{KCl}$, provided that the K_m value within brackets is in error as has been assumed [19].

The ratio of the mean activity coefficients of the electrolytes lithium chloride and potassium chloride with hydrated ions is also plotted in Fig. 1. These activity coefficients were calculated according to the formula [8 a]:

$$\gamma_{\pm}^{h} = \gamma_{\pm} (1 - 0.018\, nm)\, a_w^{n/\nu} \qquad (4)$$

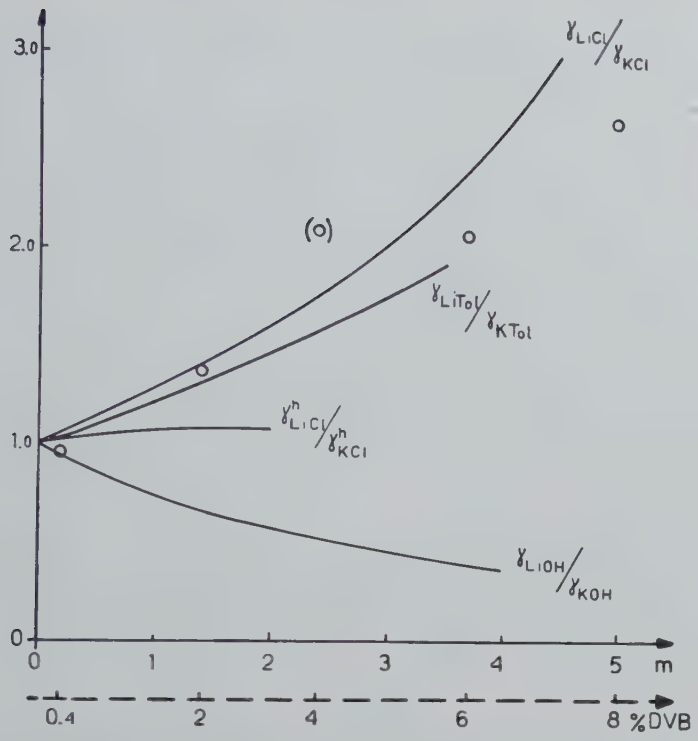

Fig. 1. The selectivity coefficient (circles) of the equilibrium K^+–Li^+,

$$K_m = m'_{Li}\, m''_K / m''_{Li}\, m'_K,$$

in a PSS ion exchanger [17] (the point within brackets is considered as uncertain [19]) and the ratios of the mean activity coefficients [18] of some electrolytes as a function of the molality. The lower abscissa axis gives the DVB content of the ion exchanger that corresponds to various molalities according to Gregor. Superscript h indicates hydrated salt.

where $\gamma^h_\pm$ is the mean activity coefficient of hydrated electrolyte
$\gamma_\pm$ the stoichiometric mean activity coefficient
n the number of molecules of water of hydration for the electrolyte according to Stokes and Robinson [8 a]
m the stoichiometric molality
ν the number of ions produced by dissociation of one molecule of the electrolyte
a_w the water activity in a m molal solution of the electrolyte.

a_w was obtained from values of the osmotic coefficient, ϕ, tabulated by Stokes and Robinson [18], according to the definition:

$$\nu\phi = -\frac{55.51}{m} \ln a_w.$$

Since Gregor considers the volumes of the hydrated ions he ought to have used the activity coefficients of the hydrated ions. On doing this, the result is much in favor of Gregor's theory as seen from Fig. 1. This is also in perfect agreement with the above-mentioned comparison by Boyd and Soldano. The curve for the ratio of the activity coefficients of the hydrated salts does not go further than 2 M (Fig. 1) since the value of the constant n for lithium chloride is not valid at higher concentrations [8a]. For higher concentrations, it is necessary to make n variable, $n = fn(m)$, but this would effectively remove the advantages of the Bjerrum-Harned-Stokes-Robinson theory in connection with ion exchangers (cf. the DVB scale in Fig. 1).

The lack of agreement between the ratio of the activity coefficients of the chlorides and the selectivity coefficient is not especially surprising. The too low value, particularly at high concentrations, of the ratio of the activity coefficients of the p-toluenesulfonates compared with K_m is probably due to a stronger interaction of the van der Waals type between the anions in the lithium salt than in the potassium salt. Cf. also the much lower values of γ_{LiTol} and γ_{KTol} compared with γ_{LiCl} and γ_{KCl} [18].

From the preceding discussion it is obvious that the selectivity coefficient cannot be accounted for by using the ratio of the activity coefficients of a salt pair with corresponding cations and a common anion. This is still more true if the common anion is chosen completely arbitrarily as seen from the discrepancy between the selectivity coefficient for the Li^+–K^+ equilibrium and the ratio of the activity coefficients of lithium and potassium hydroxides, $\gamma_{\mathrm{LiOH}}/\gamma_{\mathrm{KOH}}$, Fig. 1.

Summary

The thermodynamical equilibrium relations derived in part I of this series are used to discuss the relative contributions of the formal osmotic free energy—i.e. the product of swelling pressure and volume—and the formal ionic interaction free energy—i.e. RT times the logarithm of the ratio of the activity coefficients in the ion exchanger phase. These quantities depend strongly on the choice of ionic components, especially on whether hydrated or unhydrated ions are considered.

It is pointed out, using K^+–Li^+ as an example, that the ion exchange equilibrium between a pair of cations cannot be predicted from merely the ratio of the mean activity coefficients of a salt pair with the same cations and an arbitrarily chosen, common anion.

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REFERENCES

1. Holm, L. W., Arkiv Kemi *10*, 151 (1956).
2. Gregor, H. P., J. Am. Chem. Soc. *73*, 642 (1951).
3. Glueckauf, E., Proc. Roy. Soc. *A 214*, 207 (1952).
4. Boyd, G. E. and Soldano, B. A., Z. Elektrochem. *57*, 162 (1953).
5. Owen, B. B. and Brinkley, Jr., S. R., Chem. Rev. *29*, 461 (1941).
6. Soldano, B., Larson, Q. V., and Myers, G. E., J. Am. Chem. Soc. *77*, 1339 (1955).
7. Soldano, B. and Chesnut, D., J. Am. Chem. Soc. *77*, 1334 (1955).

8. HARNED, H. S. and OWEN, B. B., The Physical Chemistry of Electrolytic Solutions. Reinhold Publ. Corp., N.Y. 1950; (a) p. 606 f., (b) p. 562.
9. PEPPER, K. W., REICHENBERG, D., and HALE. D. K., J. Chem. Soc. *1952*, 3129 (1952).
10. REICHENBERG, D., Research *6*, No. 2, p. 9 S (1953).
11. REICHENBERG, D., PEPPER, K. W., and MCCAULEY, D. J., J. Chem. Soc. *1951*, 493 (1951).
12. REICHENBERG. D. and MCCAULEY, D. J., J. Chem. Soc. *1955*, 2741 (1955).
13. BOCKRIS, J. O'M., Quart. Rev. *3*, 173 (1949).
14. GLUECKAUF, E. and KITT, G. P., Nature *171*, 1010 (1953).
15. GLUECKAUF, E. and KITT, G. P., Proc. Roy. Soc. *A 228*, 322 (1955).
16. PEPPER, K. W. and REICHENBERG, D., Z. Elektrochem. *57*, 183 (1953).
17. GREGOR, H. P. and BREGMAN, J. I., J. Coll. Sci. *6*, 323 (1951).
18. STOKES, R. H. and ROBINSON, R. A., Trans. Farad. Soc. *45*, 612 (1949).
19. BREGMAN, J. I., Ann. N.Y. Acad. Sci. *57*, 125 (1953).

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On the formation and structure of cadmium peroxides

By Nils-Gösta Vannerberg

With 1 figure in the text

The system cadmium ion – hydrogen peroxide has been reinvestigated. Only one solid phase with variable composition was found. The limiting compositions were $Cd(O_2)_{0.74}(OH)_{0.52}(H_2O)_{2.46}$ and $Cd(O_2)_{0.88}(OH)_{0.24}$. The structure of the phase with the last-mentioned composition has been studied. It proved to be isomorphous with pyrite, with $a = 5.273 \pm 0.003$ Å. In this phase small amounts of superoxide were also detected.

Such experiments as have been reported in the literature regarding the production of pure cadmium peroxide have given rise to products which might possibly have been double salts consisting of peroxides and hydroxides. By treating cadmium hydroxide with weak solutions of hydrogen peroxide Kuriloff (1) obtained a product with the composition $CdO_2Cd(OH)_2$. In a similar manner, with 30 % hydrogen peroxide solution, Eijkmann (2) got a product with a composition corresponding to the formula $4CdO_2Cd(OH)_2$. By adding an excess of 30 % hydrogen peroxide to a weakly ammoniacal cadmium sulfate solution Teletow (3) arrived at a product which he supposed to be $5CdO_2CdO3H_2O$.

The purpose of the present investigation has been to find out which phases are formed by precipitating cadmium salt solutions with hydrogen peroxide. Besides, it was of interest to establish whether disproportionating to oxide and superoxide occurs as has been proved to be the case with calcium peroxide (4).

After a few preparatory syntheses the following one was found to be well adapted to its purpose.

Cadmium nitrate was dissolved in hydrogen peroxide solutions of varying strength and temperature. The concentration of cadmium was in all cases 0.130 *M*, while three concentrations of hydrogen peroxide (0.20, 2.5 and 9.9 *M*) were chosen.

To 50 ml of a cadmium nitrate–hydrogen peroxide solution, 15 ml 0.10 *M* sodium hydroxide solution were added. This was done at 20°C and at 80°C. In this way six samples were obtained. These were analysed with respect to cadmium (Cd^{2+}), peroxide (O_2^{2-}) and superoxide (O_2^-).

The proportion of peroxide was determined by titration with potassium permanganate and subsequent correction for the amount of superoxide. The proportion of superoxide was determined gasometrically according to Kleinberg (5). Cadmium was determined by electrolysis, whilst the hydroxide and water content were obtained from the charge balance and by difference of weight, respectively.

From the measured percentages, which can be found in Tables 1 and 2, the following conclusions may be drawn. When the precipitation of the peroxide is carried out in weak solutions of hydrogen peroxide, a phase is formed which consists of about

Table 1. 20°C.

		H_2O_2 concentration at equilibrium		
		0.15 *M*	1.9 *M*	7.6 *M*
CdO_2	w. %	58.2	75.2	81.4
	mol. %	21.4	47.4	65.4
$Cd(OH)_2$	w. %	16.7	16.4	13.2
	mol. %	7.5	10.2	10.4
H_2O	w. %	24.1	8.4	3.6
	mol. %	71.1	42.5	23.3
$Cd(O_2)_2$	w. %	—	—	1.6
	mol. %	—	—	1.0
Approx. formula		$Cd(O_2)_{0.74} \cdot (OH)_{0.52} \cdot (H_2O)_{2.46}$	$Cd(O_2)_{0.82} \cdot (OH)_{0.36} \cdot (H_2O)_{0.74}$	$Cd(O_2)_{0.85} \cdot (OH)_{0.28} \cdot (O_2^-)_{0.02} \cdot (H_2O)_{0.30}$
Colour		yellow	gray-yellow	gray

Table 2. 80°C.

		H_2O_2 concentration at equilibrium		
		0.15 *M*	1.9 *M*	7.6 *M*
CdO_2	w. %	73.9	85.7	85.4
	mol. %	62.7	71.4	86.0
$Cd(OH)_2$	w. %	23.5	10.4	12.5
	mol. %	19.7	8.6	12.3
H_2O	w. %	2.6	2.9	—
	mol. %	17.6	19.3	—
$Cd(O_2)_2$	w. %	—	1.0	2.1
	mol. %	—	0.7	1.7

two and a half times as much peroxide as hydroxide. The composition of this product is similar to the composition of those mentioned in the references (1) and (3). Besides, the precipitates contained considerable quantities of water, probably in the form of a gel. At higher concentrations of hydrogen peroxide and higher temperatures the precipitates approach the composition $Cd(O_2)_{0.88} \cdot (OH)_{0.24}$ that may be formulated as $7CdO_2 \cdot Cd(OH)_2$ (Tables 1 and 2). In the latter product there are also small impurities of superoxide, as could be shown at examination for magnetic properties according to the Gouy method.

The substance $Cd(O_2)_{0.88} \cdot (OH)_{0.24}$ decomposes violently when rapidly heated, but on slow heating to 100°C the peroxide disproportionates in the same way as has been shown in connection with calcium peroxide according to the formula

$$3CdO_2 + 2H_2O \rightarrow 2Cd(OH)_2 + Cd(O_2)_2.$$

More details of this work will be published at a later date.

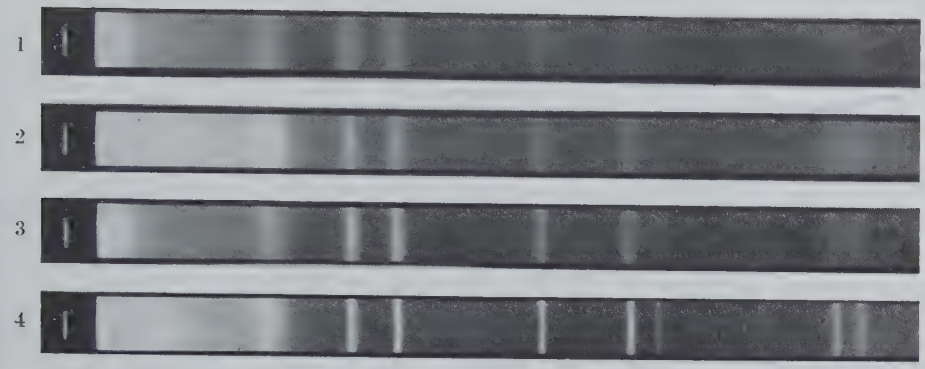

Fig. 1. Guinier photographs of products precipitated from solutions containing cadmium ions and hydrogen peroxide.

1. 20°C. 0.15 *M* H_2O_2
2. 20°C. 1.9 *M* H_2O_2
3. 20°C. 7.6 *M* H_2O_2
4. 80°C. 7.6 *M* H_2O_2

The products mentioned in Tables 1 and 2 were examined by the aid of powder photographs (Guinier method). In all cases the same pattern appeared. Only the diffraction breadths were different, so that samples containing much hydroxide and water have considerably wider lines than those rich in peroxide. No trace of cadmium hydroxide lines appeared. See Fig. 1. Consequently it seems that, although the compostition varies between $Cd(O_2)_{0.74} \cdot (OH)_{0.52} \cdot (H_2O)_{2.46}$ and $Cd(O_2)_{0.88} \cdot (OH)_{0.24}$, in the products formed by hydrogen peroxide and cadmium ions there is only one crystalline phase. The water is probably bound in the form of a gel, while, on the other hand, substitution of hydroxide ions in the peroxide lattice is not impossible. The varying diffraction breadths may be explained in terms of varying crystallite sizes.

In connection with the observation mentioned above it was of interest to investigate whether any intermediate phase, e.g. cadmium hydroxide, is formed by reaction of hydrogen peroxide with solid cadmium oxide. A sample of cadmium oxide was treated with 30 % hydrogen peroxide for a short time. On a powder photograph of the substance formed, there appeared, in addition to lines of cadmium oxide, lines from the above-mentioned phase.

A structural examination was undertaken by the aid of Guinier photographs of the precipitate obtained from a concentrated solution of hydrogen peroxide, i.e. $Cd(O_2)_{0.88} \cdot (OH)_{0.24}$. $\sin^2\theta$ for all the reflexions (Table 3) can be indexed on the assumption of a cubic elementary cell with an edge length of 5.273 Å. According to the experimentally determined density, 5,45 g/cm^3, the number of formula units per elementary cell is only 3.3. Bearing in mind that density determinations generally give rather low values and that these compounds decompose yielding oxygen, it is probable that there are four formula units in the elementary cell. If four formula units are to be found in the elementary cell 41.4 % of the available space is occupied by atoms. In pyrite (FeS_2), which turned out to be isomorphous with the phase investigated, the same value is 40.6 %.

On the photographs no absence is observed for the reflections of the type *hkl*. But further it may be pointed out that those reflections which do not belong to a

Table 3.

hkl	$10^4 \sin^2\theta_{obs}$	$10^4 \sin^2\theta_{calc}$
111	640	641
200	856	855
210.120	1066	1068
112	1286	1282
220	1709	1710
221	~1910	1923
113	2353	2351
222	2568	2564
320.230	~2786	2778
321.123	2992	2992
400	3421	3419
410.140	~3628	3633
114	3846	3847
331	4060	4060
420.240	4273	4274
421.124	—	4488

$a = 5.273 \pm 0.003$ Å

face-centered lattice are very weak. The reflections $0kl$ are found only for $k = 2n$. This is characteristic for the space group No. 205 T_h^6–Pa3.

It was found that the four cadmium atoms could be placed in the positions $\frac{1}{2}\frac{1}{2}\frac{1}{2}$; $\frac{1}{2}00$; $0\frac{1}{2}0$; $00\frac{1}{2}$ ($4b$) and the eight oxygen atoms in

$$\pm(xxx;\ \tfrac{1}{2}+x\,\tfrac{1}{2}-x\,x;\ x\,\tfrac{1}{2}+x\,\tfrac{1}{2}-x;\ \tfrac{1}{2}-x,\ x,\ \tfrac{1}{2}+x) \qquad (8b)$$

The intensity calculations were made according to a method given by E. Hellner (6).

The x-parameter was varied systematically. The best agreement between calculated and observed intensities was obtained with a parameter value of 0.082 (Table 4). This value was calculated on the assumption that the position ($8b$) is occupied only by peroxide ions. However, as mentioned above, it is not improbable that some of the peroxide ions have been replaced by hydroxide ions. If this were taken in account, a slight change in the value of the parameter would probably result. But the dominance of the cadmium atoms is too great for the oxygen parameter value to be determined with certainty and accordingly nothing can here be said about the occurrence of hydroxide ions in this lattice. The parameter value mentioned corresponds to an oxygen–oxygen distance in the same peroxide group of 1.50 Å. The uncertainty may be of the order 0.1. The distance of oxygen to cadmium will then be 2.29 ± 0.02 Å. According to Pauling the calculated value for an ionic bond is 2.33 and for a covalent bond 2.23 Å. In spite of the fact that both the oxides and hydroxides of calcium and cadmium are isomorphous their peroxides are not. CaO_2 is isomorphous with calcium carbide, while CdO_2 crystallizes with the pyrite type structure. This indicates, in agreement with the greater electron negativity and the 18-electron structure of cadmium, a somewhat more covalent character of the bonds in the CdO_2 structure.

Finally it may be added that an investigation of peroxides and superoxides of

Table 4.

hkl	I_{obs}	I_{calc}		
		$x = 0.070$	$x = 0.082$	$x = 0.090$
111	65	61	65	68
002	70	75	71	67
210.120	1.0	0.7	1.1	1.5
112	1.2	0.9	1.2	1.5
220	45	48	45	43
221	0.1	0.3	0.3	0.3
113	50	46	50	52
222	14	17	16	15
320.230	0.5	0.6	0.8	0.8
321.123	0.7	0.8	0.9	1.1
004	7	7	6	5
410.140	0.3	0.1	0.1	0.1
114	0.4	0.2	0.2	0.2
331	21	24	24	24
420.240	19	21	22	22
421.124	0	0.0	0.0	0.0

magnesium, zinc and mercury is going on. It has been proved that ZnO_2 and one phase of HgO_2 are isomorphous with CdO_2 with edge lengths of the unit cells 4.87 and 5.32 Å respectively.

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REFERENCES

1. Kuriloff, Ann. chim. phys. (6) *23*, 429 (1891).
   —— Compt. rend. *137*, 618 (1903).
   Gmelins Handbuch der anorganischen Chemie, 8. Aufl., Leipzig–Berlin, 1924. Vol. *33*, p. 72.
2. Eijkmann, Chem. Weekbl. *2*, 295 (1905).
   Gmelins Handbuch der anorganischen Chemie, 8. Aufl., Leipzig–Berlin, 1924. Vol. *33*, p. 72.
3. Teletow, I., Chem. Zentr. *15* I, 1799 (1911).
4. Brosset, C. and Vannerberg, N.-G., Nature *177*, 238 (1956).
5. Kleinberg, J. and Seyb, E., J. Am. Chem. Soc. *23*, 115 (1951).
6. Hellner, E., Z. Krist. *106*, 122–45 (1954).

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On the thermodynamics of ion exchange equilibria

III. On the prediction of ion exchange equilibria

By Lennart W. Holm

Introduction

Starting from the thermodynamical equations for an ion exchange equilibrium derived in part I [1] of this series of papers, the relative importances of the activity coefficients in the ion exchanger phase and of the swelling pressure-partial molal volume product were discussed in part II [2]. In the latter paper it was also pointed out especially that the relative distribution of the two ions between an ion exchanger and a surrounding solution cannot be confidently predicted by a comparison of the mean activity coefficients of two electrolytes composed of the same cations and an arbitrary, common anion. It is, nevertheless, of value to be able to estimate qualitatively the relative affinities of ions for ion exchangers. A method for such a prediction applicable to simple ions is introduced here. The more desirable quantitative calculation necessitates the knowledge of relevant activity coefficients, partial ionic volumes and swelling pressure in the ion exchanger phase. The evaluation of these quantities will be discussed.

Qualitative prediction of ion exchange equilibria

It is commonly stated, with reference to the alkali metals, that the ion having the largest "hydrated" ionic radius (or volume) has the lowest affinity for the ion exchanger. This statement is complemented with another stating that the ion having the smallest crystallographic radius has the largest hydrated radius (due to increased coulombic interaction with the water dipoles). Thus the affinity of the alkali metals for the ion exchanger should increase in the order, $Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$. This is in fact in agreement with experimental results for polystyrenesulfonic acid (PSS) ion exchangers but quite contrary to those for polycarboxylic acid ion exchangers [3]. Similarly it seems difficult to explain in this manner for example the high affinity of the perchlorate ion [4] for strong anion exchangers. Bregman [3] has explained the behavior of the alkali metal ions on weak cation exchangers, like polycarboxylic acid, by the use of the Robinson-Harned-Stokes hypothesis [5a] of "localized hydrolysis". This hypothesis states that the protons (of near by water molecules) repelled by the positive ion possess a certain tendency to form bonds with proton acceptors such as hydroxyl or acetate ions present in the solution. The strength of the bond should then depend on

the strength of the electric field around the cation, i.e. it should decrease from Li^+ to Cs^+. Thus the affinity for a carboxylic acid exchanger should decrease according to: $Li^+ > Na^+ > K^+$.

The theory of Gurney.—Robinson *et al.* used the hypothesis of "localized hydrolysis" to explain the decrease of the activity coefficients of the alkali hydroxides from cesium hydroxide to lithium hydroxide which is opposite to the case for the alkali halides (with the exception of the fluorides). Gurney's theory [6] with the conceptions of "order-producing" and "order-destroying" ions seems, however, to be more attractive and profitable. Gurney starts from the semi-empirical postulate that there is a certain degree of order among the molecules of water at room temperature and that the time average number of nearest neighbours of a water molecule is about four, arranged tetrahedrally and advantageously from the electrostatic point of view. When introduced into the water, an ion will interfere with this structure in a way depending on the charge and size of the ion. Gurney introduced the name co-sphere for the water layer surrounding an ion and defined it as "the little spherical portion of solvent which encloses the ion and which is somewhat modified by the presence of the ion". In the co-sphere, all those solvent molecules are included which give any measurable contribution to the property being studied. If an ion of high field strength, e.g. Li^+, is introduced into water, it will have a strong ordering effect on the water molecules (dipoles) in the co-sphere resulting in a higher degree of order than that previously present in the water; such an ion is therefore called order-producing. If on the other hand the ion is so large that it disturbs the water structure due to geometrical reasons, the result is a breakdown of the water structure of the co-sphere and in addition the weaker electrostatic field will be uncapable of ordering the water dipoles to any noteworthy extent; such an ion, e.g. Cs^+, is consequently called order-destroying. Using these conceptions, Gurney has successfully discussed the electric conductivity and viscosity of electrolyte solutions and correlated them with partial molal ionic entropies in water. The treatment of more concentrated solutions is of special interest in connection with ion exchangers. In a concentrated solution where the co-spheres of two ions are forced to overlap, the Coulomb force between the ions will, according to Gurney, be superimposed by a repulsive force of short range if the free energy is greater in this state than when the co-spheres do not overlap, i.e. if the co-spheres are of different character from the point of view of order. Conversely, a superimposed attractive force is obtained if the state of order is the same.

With regard to the alkali metals, we have the strongly order-producing lithium ion and then the sodium ion, which is less order-producing, after which the order-destroying power increases from the potassium ion to the cesium ion. Consequently the interaction between these ions and anyone of the order-destroying halide ions Cl^-, Br^- or I^- will increase from Li^+ to Cs^+, i.e. the activity coefficient decreases from Li (Cl, Br or I) to Cs (Cl, Br or I). This may be expressed in general terms as follows: The activity coefficient decreases when we go from ions of dissimilar order–disorder character to ions of similar character. Without the necessity of using any special hypothesis like the one of localized hydrolysis mentioned above, it follows that the activity coefficients of salts of the alkali metals with order-producing anions, such as hydroxide and fluoride, will decrease from cesium to lithium—this is in agreement with experimental results [7]. From Gurney's theory, it can also be concluded that the activity coefficients of the rubidium and cesium

halides decrease from the fluorides to the iodides which is contrary to the case for the halides of the other alkali metals; this phenomenon has (according to Gurney) not been explained earlier. It must, however, be pointed out that it is not possible to draw any conclusions from Gurney's theory regarding the activity coefficients of salts of the transition metals in relation to those of for example alkali metal salts. The reason for this is the strong quantum-mechanical forces superimposed on the Coulomb forces in the case of the transition metals.

Application of Gurney's theory to ion exchange equilibria.—For the application of Gurney's theory to ion exchange equilibria, it is of fundamental interest to know if the functional group of the ion exchanger is order-producing or order-destroying. Gurney has stated a correlation between the strength of an acid and the state of order in the co-sphere around its anion: the anion of a weak acid is order-producing while that of a strong acid is order-destroying. Note that the consequence of this is the same as that of the hypothesis of localized hydrolysis. In agreement with this, the perchlorate ion, for example, is strongly order-destroying and the acetate ion order-producing. The former result could be expected from the large size of the perchlorate ion and from the even charge distribution, due to resonance, over the four oxygen atoms. It might be a little more difficult to understand the order-producing character of the acetate ion—exemplified by among other things the decrease of the activity coefficients of the alkali acetates from cesium acetate to lithium acetate [7]—since this indicates that there is no resonance in this ion in aqueous solution and that the charge is localized to one of the oxygen atoms of the carboxylic group. According to Gurney, the reason for this is that it is "extremely unlikely that the solvent dipoles that are in contact with the two oxygen atoms happen to produce identical polarizations, and provide for the negative charge states of equal energy between which the electron can resonate". If this argument is accepted, a possible explanation of the fact that the same is not true for the perchlorate ion is the greater resonance energy of the latter.

From the preceding, it can be seen that the sulfonate ion is order-destroying in agreement with the decrease of the activity coefficients of the alkali para-toluenesulfonates from LiTol. The lower values of these coefficients in comparison with the ones for the corresponding alkali chlorides must however not be taken as an indication of a smaller order-destroying power for the sulfonate ion compared with that for the chloride ion. The displacement towards lower values for the sulfonates depends namely, as pointed out before, on the van der Waals interaction between the anions. In part II, Fig. 1 [2], it was shown that the ratio of the activity coefficients for lithium chloride and potassium chloride, contrary to the corresponding ratio for lithium hydroxide and potassium hydroxide, gives a correct indication of the selectivity coefficient for the K^+–Li^+ equilibrium in a PSS ion exchanger. Expressed in Gurney's terms, the reason for this is that the chloride ion like the sulfonate ion is order-destroying while the hydroxyl ion is order-producing. Any quantitative agreement is on the other hand merely fortuitous.

Analogously to the acetate ion, the carboxylic ion is generally order-producing. Consequently the relative affinities of the alkali ions for carboxylic acid ion exchangers are the reverse of those of the same ions for PSS ion exchangers. The conditions are analogous for anion exchangers. The strongly basic anion exchangers consist of polystyrene with quaternary ammonium groups (e.g. $-CH_2N^+(CH_3)_3$ in Dowex 1) which, as expected (large group with the charge on the nitrogen atom in the centre), are order-destroying. This is obvious from, among other things,

the increase of the affinities of the halide ions from the order-producing fluoride ion to the strongly order-destroying iodide ion [4] and also from the low affinity of the order-producing acetate ion and the high affinity of the order-destroying perchlorate ion.

From the order-disorder theory it follows that an ion exchanger sorbs less water when it is saturated with an ion of the same order–disorder character as its functional group than when the counter ion is of opposite character—this is in agreement with the results of Boyd and Soldano [8] and those of Soldano and Larson [9]. It is also interesting to note the statement of the former authors that the decrease of the osmotic coefficients of Dowex 1 saturated with various anions in the order $F^- > OH^- > Cl^- > Br^- > > ClO_4^-$ is contradictory to the case for the corresponding lithium, sodium, and potassium salts but in agreement with the behavior of the cesium salts.

As one of the proofs of the validity of the pressure-volume theory of Gregor [10] (see also part II [2]), Bregman [3] gives the relation between the volumes of an ion exchanger saturated with various ions. Thus, the volume of a PSS ion exchanger decreases according to $Li^+ > Na^+ > K^+$. At least in those cases where the ions are almost equally large, the volume difference between two different ionic forms of the ion exchanger depends mainly, however, on the difference in interaction between the ion exchanger and the ions and on the difference produced in the amount of sorbed water, i.e. the volume difference is a secondary phenomenon with the same primary cause as the differing affinities (or activity coefficients). In the thermodynamical equilibrium equation (cf. eqn. (2) below), a greater interaction with the resin of one ion, say B, compared to the other may give rise to both a smaller activity coefficient and a smaller volume; both effects lead to a rise in K_m, the selectivity coefficient. The strong dependence of the volume on the interaction is shown by the results obtained by Boyd and Soldano [8] indicating that the equivalent volume of the anion exchanger Dowex 1 (6 % DVB) is 431 ml in fluoride form and 300 ml in perchlorate form in spite of the fact that the equivalent volumes of the nonhydrated ions are: F^- 6.3 and ClO_4^- 55.0 ml. Similar results have been obtained by Gregor, Belle, and Marcus [11, 12] for Dowex 2. The measurements made by Gregor *et al.* [13] on polymethacrylic acid ion exchanger are consistent with the above. Thus, this ion exchanger, having an order-producing functional group, has a larger volume in potassium form than in lithium form, which is contrary to the case for the order-destroying PSS ion exchanger. From Gurney's theory, it is in fact clear that the volume should decrease the more the co-spheres can overlap which is in complete agreement with the above.

Quantitative prediction of ion exchange equilibria

In order to make a quantitative calculation of the selectivity coefficient, K_m, defined by

$$K_m = \left(\frac{m'_A}{m''_A}\right)^{z_B} \cdot \left(\frac{m''_B}{m'_B}\right)^{z_A} \tag{1}$$

where m'_A and m''_A are the molalities of the ion A in phase $'$ and $''$, m'_B and m''_B the corresponding quantities for B, and z_A and z_B the valences of A and B, from for instance the equation (eqn. (2 b) in part II [2]):

$$RT \ln K_m = RT \ln \frac{\gamma'_B (m'_A, m'_B)}{\gamma'_A (m'_A, m'_B)} - RT \ln \frac{\gamma''_B (m''_A, m''_B)}{\gamma''_A (m''_A, m''_B)} + \\ + \pi \{v_A (m''_A, m''_B) - v_B (m''_A, m''_B)\} \quad (2)$$

we need to know the partial molal volumes of the nonhydrated ions, v_A and v_B, the swelling pressure, π, in the ion exchanger phase, the activity coefficients in the resin phase γ''_A and γ''_B (both at atmospheric pressure) and those in the external solution γ'_A and γ'_B. For further explanation of symbols see part I [1].

In the discussion of part II [2], it was pointed out that the volume difference $\{v_A (m''_A, m''_B) - v_B (m''_A, m''_B)\}$ can be approximated by the same difference at infinite dilution $(v^0_A - v^0_B)$ as suggested by Soldano and co-workers [14, 15] and justified by the fact that the contribution of the pressure-volume term to the selectivity coefficient is small compared with that of the ratio of the activity coefficients in the ion exchanger phase. In a few cases, a fair estimate of the volume difference at high concentrations can be made by using published data [5 b]. This was done by Glueckauf [16].

The swelling pressure.—We start from the equilibrium condition for the distribution of an uncharged component between the ion exchanger phase ($''$) and the surrounding solution ($'$) (cf. eqn. (4) part I [1]) which for water can be written

$$\mu''_w (p'', m'') = \mu'_w (p', m') \quad (3)$$

where $\mu''_w (p'', m'')$ is the chemical potential of water in phase $''$ at the pressure p'' and molality m''. Taking pure water at a pressure of 1 atmosphere as standard state in both phases, i.e. $\mu''^0_w (1) = \mu'^0_w (1)$, and assuming the pressure above the external solution to be constant and equal to 1 atmosphere, we obtain

$$a''_w (p'', m'') = a'_w (1, m'). \quad (3\,a)$$

The pressure dependence of the activity follows from (cf. eqn. (13) part I):

$$RT \left(\frac{\partial \ln a''_w (p'', m'')}{\partial p} \right)_{m''} = v''_w (p'', m'') \quad (4)$$

which integrated from 1 to p'' gives:

$$RT \ln \frac{a''_w (p'', m'')}{a''_w (1, m'')} = \int_1^{p''} v''_w (p'', m'')\, dp. \quad (5)$$

v_w is, however, only very slightly dependent on pressure—a pressure increase from 100 to 1000 atmospheres causes a decrease in v_w of only 4 per cent [8]. Thus eqn. (5) can be written:

$$\pi v''_w (m'') = RT \ln \frac{a''_w (p'', m'')}{a''_w (1, m'')} \left(= RT \ln \frac{a'_w (1, m')}{a''_w (1, m'')} \right). \quad (6)$$

Among the deductions of this formula [8, 16, 17], given earlier, the one by Boyd and Soldano [8] is the most clear and most satisfactory especially from the ion exchange point of view. From eqn. (6), it follows that a comparison of the water

activity $a''_w(p'', m'')$ in an ion exchanger with a given degree of crosslinking (corresponding to the pressure p'') with the water activity $a''_w(1, m'')$ of a strain-free ion exchanger of the same composition, m'', gives a value for $\pi v''_w(m'')$. The water activities needed for this calculation can be obtained from water sorption isotherms—amount of water sorbed per amount of ion exchanger as a function of the water activity (relative humidity)—for ion exchangers with varying degrees of crosslinking [10]. Such isotherms have been established by isopiestic measurements for cation exchangers as well as for anion exchangers saturated with various ions [8, 16, 18, 19, 20].

It has just been mentioned that the determination of the swelling pressure necessitates a knowledge of the water activity of a strain-free ion exchanger polymer. At first sight one might consider a linear non-crosslinked polymer to best satisfy this claim. Measurements by Gregor *et al.* [21, 22] on linear polystyrenesulfonic acid have however shown that at low relative humidities this acid and its salts sorb considerably less water than the corresponding crosslinked compounds. It seems that the reason for this phenomenon is that the linear polymer chains aggregate side by side and are held together by van der Waals forces. This increase of polymer–polymer interaction results in a decrease of the interaction between polymer and water, i.e. the water activity at constant water content increases. On the introduction of a certain amount of DVB, the polymer chains are kept apart by the crosslinks and thus the polymer–polymer interaction decreases, the polymer–water interaction increases and, consequently, the water activity decreases [21]. Boyd and Soldano [8] drew the same conclusion from the fact that the volume of a given ion exchanger at the swelling pressure $\pi = 0$—obtained by extrapolation of a plot of the swelling pressure versus the ion exchanger volume, which is a straight line (cf. below)—is greater than the volume of the same ion exchanger free from water. Thus, since the configurations of a non-crosslinked polymer and a crosslinked polymer are not the same, the water activity of the former cannot be used for calculating the swelling pressures in crosslinked polymers. Judging from Gregor's *et al.* results [20], there is, as could be expected, no discontinuity in the transition from one configuration to the other on introduction of DVB. On the contrary, the lower water sorption depending on the higher polymer–polymer interaction at low humidities seems to remain to some extent also at rather high degrees of crosslinking; e.g., according to Gregor, even at such a high relative humidity as 0.4, a H^+-saturated PSS ion exchanger with 8 per cent DVB sorbs almost 10 per cent less water than the same exchanger with 10 per cent DVB. It should however be remembered that this is not in agreement with results obtained by other authors [8, 16, 19]. The discrepancy may, however, depend to a large extent on the experimental difficulties; especially the long time needed for equilibration and the uncertainty of the moisture content determination particularly at low moisture contents and low degrees of crosslinking.

When the relative humidity approaches a value of one, a polymer with 0.5–2 % DVB sorbs approximately the same amount of water as a linear polymer, i.e. $\pi \cong 0$, but the sorption is in accordance (for relative humidities above 0.6) with the sequence of sorption for polymers with higher degrees of crosslinking, i.e. the configuration of the 2 % DVB polymer is the same as that for higher DVB contents. Consequently ion exchangers with DVB contents of 0.5–2 per cent can be used as standards for the determination of the swelling pressure [8, 16, 22]. To calculate π from the product $\pi v''_w(m'')$ thus obtained (cf. above), the partial

molal volume of water $v_w''(m'')$ must be known at the composition m'' under consideration. The dependence of $v_w''(m'')$ on the composition is, however, rather small and, at least at relative humidities above ~ 0.5, $v_w''(m'')$ may be replaced by the molal volume of pure water independent of the ion saturating the exchanger [8, 18].

The swelling pressure depends on many factors apart from the type of the ion exchanger. As expected, π increases with the degree of crosslinking, the moisture content and the size of the (in relevant cases "hydrated") ion [8, 16, 22]. These investigations have shown that, at higher moisture contents, the ion exchanger obeys Hooke's law, i.e. there is a linear relation between π and the (external) equivalent volume of the ion exchanger. For a given ion exchanger with a given degree of crosslinking, this relationship is independent of the moisture content and the ion saturating the exchanger [8, 10, 16, 22]. For this reason, the numerous volume measurements made on ion exchangers and the work expended on finding reproducible and adequate methods [23, 24, 25] are increased in value.

In the study of ion exchange equilibria, we are interested in the swelling pressure of an ion exchanger containing two ions simultaneously. Soldano and Chesnut [15] assumed the pressure to vary linearly with the equivalent fraction which is, as they pointed out, true if the equivalent volume also does so. This supposition may be true for ions of the rare gas type, i.e. alkali ions in strong cation exchangers and halide ions in strong anion exchangers, as illustrated by the example Na^+–H^+ in a PSS ion exchanger [24]. It can, however, only be regarded as a poor approximation for large, e.g. organic, ions; cf. K^+–$(CH_3)_4N^+$ [23] and Cl^-–CH_3COO^- [11].

The activity coefficients.—The activity coefficients in the external solution are of course independent of the ion exchanger and are therefore without interest in the present discussion. The activity coefficients in the ion exchanger phase can be calculated if the corresponding osmotic coefficients are first determined. The osmotic coefficient, ϕ'', in a resin of molality m'' and at a pressure of one atmosphere is defined by:

$$\nu\phi''(1,m'') = -\frac{55.51}{m''}\ln a_w''(1,m'') \tag{7}$$

where ν is the number of osmotically active ions per mole exchanger. When the swelling pressure of the resin is $\pi = p'' - 1$, we obtain for the osmotic coefficient (still at one atmosphere) as a function of the actual water activity $a_w''(p'',m'')$ in the ion exchanger by combination with eqn. (6):

$$\nu\phi''(1,m'') = -\frac{55.51}{m''}\ln a_w''(p'',m'') + \frac{\pi}{RTm''} \tag{8}$$

since $v_w \cong 0.018 = 1/55.51$. Corresponding values for a_w and m are obtained from the water sorption isotherms previously described. Those authors [8, 9, 16] who have studied the dependence of ϕ on the molality in the ion exchanger phase have worked with ion exchangers with low degrees of crosslinking (0.5 per cent DVB). In these exchangers, the swelling pressure was found to be approximately zero provided that the molality is not too low. In such cases, eqn. (8) can be approximated by:

$$\nu\phi''(1,m'') = -\frac{55.51}{m''}\ln a_w''(p'',m'') \quad p''\cong 1. \tag{8a}$$

Glueckauf [16] and Boyd and Soldano [8] have applied eqn. (8a) and in a diagram of $\nu\phi = fn(m)$ extrapolated to $m=0$. They considered that they obtained $\nu\phi_{m=0}=1$ from which $\nu=1$ since $\phi_{m=0}=1$ according to its definition. This conclusion was drawn despite the fact that Glueckauf derived the complete eqn. (8) in which he however immediately neglected the swelling pressure term and although Boyd *et al.* pointed out in a foot-note that $\nu\phi=0$ at a molality corresponding to maximum swelling. The impossibility of such an extrapolation giving a ϕ-value of one at infinite dilution is easily seen from the fact that a crosslinked exchanger can never become "infinitely diluted". Even if the extrapolation mentioned above does not justify the conclusion that $\nu=1$, this value seems to hold for ion exchangers. This means that the polyion is osmotically inactive in accordance with its immobility [8, 9, 16, 18]. Among arguments for this conclusion, it may be mentioned that the curves $\nu\phi=fn(m)$ for ion exchangers saturated with various ions have the same general shape as the curves for simple salts with corresponding ions [8, 16] but deviate at low concentrations [9]. Gregor *et al.* [18] have compared for example the water sorption isotherm for hydrogen-saturated PSS ion exchanger (10 per cent DVB) with that of sulfuric acid ($\nu=2$ in concentrated solutions). They found an agreement for $\nu=1$ but not for $\nu=2$ in the ion exchanger; the same result was obtained with other degrees of crosslinking. On the other hand the sorption isotherm for linear polystyrenesulfonic acid behaves quite differently due to the increased polymer–polymer interaction discussed above, and consequently such a curve is irrelevant in this connection.

Since the osmotic coefficient is not known down to $m=0$ it is not possible to calculate the activity coefficient in the usual manner (by application of the Gibbs-Duhem equation) unless, as Gregor *et al.* [21] put it, "an awkward and arbitrary standard state" is chosen. Glueckauf [16] got round this difficulty by introducing the function:

$$\phi - 1 = -f(m) + A_{M^+} \tag{9}$$

where $f(m)$ is an unknown function of m identical for all univalent cations and A_{M^+} is a constant having a characteristic value for each ion. His results show a reasonable constancy of $A_{H^+} - A_{M^+}$ for $M^+ = Li^+$, Na^+, K^+, or Ag^+ in the range $m=5$–20. It is still not possible to obtain the absolute values for the activity coefficients but on the other hand the ratio of the activity coefficients of two different, pure ionic forms of the ion exchanger at the same concentration is easily calculated, since the unknown function $f(m)$ then cancels out. From this activity coefficient ratio, Glueckauf obtained the corresponding ratio for mixed ionic forms by application of the Harned rule [5c]:

$$\log\left(\frac{\gamma_1}{\gamma_2}\right) = \log\left(\frac{\gamma_1^0}{\gamma_2^0}\right) + \alpha_{21}m_1 - \alpha_{12}m_2 \tag{10}$$

where γ_1 and γ_2 are the mean molal activity coefficients of the electrolytes 1 and 2 in a solution of constant total molality $m = m_1 + m_2$, which is m_1 molal in 1 and m_2 molal in 2, γ_1^0 and γ_2^0 are the activity coefficients in a pure m molal

solution of 1 and 2 respectively, and α_{21} and α_{12} are the constant so-called interaction coefficients. Glueckauf then makes the assumption that $\alpha_{12} = -\alpha_{21}$ which is however permissible only in exceptional cases even for ordinary water solutions. It should be noticed that the activity coefficients obtained in this manner are valid at one atmosphere since they are calculated from osmotic coefficients determined according to eqn. (8a). Consequently eqn. (2) applies to the ion exchange equilibrium and Glueckauf used this formula to calculate the selectivity coefficients for some equilibria that had earlier been studied experimentally. The agreement achieved is not so good (calculated values are 10 per cent or more too high).

A more advanced treatment along principally the same lines as Glueckauf's has been made by Soldano and co-workers [9, 14, 15]. Instead of using eqn. (9) they accounted for the concentration dependence of the osmotic coefficients by using the formula suggested by Scatchard and simultaneously assuming ϕ to be independent of the DVB content (below 0.5 % DVB) and only a function of the molality:

$$\phi = f(m) + \frac{a\,m}{1 + b\,m} \qquad (11)$$

where a and b are constants characteristic for each ion and $f(m)$ is, as in Glueckauf's treatment, an unknown function which should account for the osmotic coefficient at low concentrations. By considering the experimentally accessible part of the ϕ-curve and its extrapolation to zero molality, Soldano *et al.* show that the function $f(m)$ can be put equal to zero for anion exchangers (Dowex 1 and 2) and equal to a constant, which has the same value for all simple cations of the rare gas type, for cation exchangers. They found also that the constants a and b are interdependent and therefore ϕ can be in principle expressed by means of a one-parameter equation [9]. It is also of interest to note that they have been able to correlate a with the crystallographic radius of the ion.

In the same way as Glueckauf, Soldano *et al.* obtained an expression for the ratio of the activity coefficients of two different, pure ionic forms [14, 15]. In doing so, they point out that the $f(m)$ function is not necessarily identical for two different ions and thus the calculated activity coefficient ratio may not be the absolute value. This objection is of course also true for Glueckauf's treatment. By means of "trace selectivity" measurements (i.e. one of the ions is present in tracer amounts only: e.g. the ion 1, then $m = m_1 + m_2 \cong m_2$ and eqn. (10) is reduced to $\log(\gamma_1/\gamma_2) = \log(\gamma_1^0/\gamma_2^0) - \alpha_{12}\,m_2$) at various degrees of crosslinking, the Harned rule was shown to be invalid because of the variation in the interaction coefficients. The assumption by Glueckauf that $\alpha_{12} = -\alpha_{21}$ (cf. above) is still less valid.[1] On the contrary, the interaction coefficients were found to vary with the molality according to, for example, $\alpha_{12}(m) = \alpha_{12}^0/R_1(m)$ where α_{12}^0 is a constant and $R_1(m)$ is a function of the molality related to the molality dependence of the activity coefficient and contains as parameter only the constant b, mentioned above as entering eqn. (11) for the osmotic coefficient. In this manner, Soldano *et al.* obtained a surprisingly good agreement between calculated and experimental

[1] The inapplicability of the Harned rule is also the main reason for the worse agreement between calculated and experimental values for the selectivity coefficient obtained among others by Davies and Yeoman [26, 27].

values of the selectivity coefficients for various combinations of simple, univalent cations and halide ions. They have also found a correlation between the interaction coefficients (α_{12}^0) and the constants a and b which enter the expression for the corresponding osmotic coefficient. This means that it should be possible to predict selectivity coefficients knowing only the osmotic coefficients of the corresponding pure ion exchangers. Thus it seems as if the treatment of Soldano *et al.* is the most satisfactory osmotic treatment of ion exchange equilibria given hitherto. Their contribution must be considered to be a very good introduction to a better understanding of the quantitative treatment of the problem of ion exchange equilibria.

Summary

Gurney's theory of order- and disorder-producing ions is applied to ion exchange equilibria. Using it, the selectivity coefficients are qualitatively predicted for ions with completed main electronic shells.

The osmotic behavior of ion exchange resins and the calculation of swelling pressures and of osmotic and activity coefficients from experimental water sorption isotherms are discussed. In order to obtain the activity coefficients in the mixed ionic forms of a resin from those in the pure ionic forms, the Harned rule cannot generally be used in its simple form, i.e. with concentration-independent interaction coefficients. However, by introduction of concentration-dependent interaction coefficients Soldano *et al.* have obtained excellent agreement between calculated and experimental data for selectivity coefficients.

The author wishes to thank the Head of this Institute, Professor Manne Siegbahn, for his kind interest in this work. The author is also very much indebted to Professor Lars Gunnar Sillén and Dr. Wilhelm Forsling for their valuable criticisms on the manuscript of this and the two preceding parts in this series of papers.

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REFERENCES

1. Holm, L. W., Arkiv Kemi *10*, 151 (1956).
2. Holm, L. W., Arkiv Kemi *10*, 445 (1956).
3. Bregman, J. I., Ann. N. Y. Acad. Sci. *57*, 125 (1953).
4. Peterson, S., Ann. N. Y. Acad. Sci. *57*, 144 (1953).
5. Harned, H. S. and Owen, B. B., The Physical Chemistry of Electrolytic Solutions. Reinhold Publ. Corp., N. Y. 1950; (a) p. 383 f., (b) p. 253, (c) p. 461 f.
6. Gurney, R. W., Ionic Processes in Solution. McGraw-Hill, N. Y. 1953.
7. Stokes, R. H. and Robinson, R. A., Trans. Farad. Soc. *45*, 612 (1949).
8. Boyd, G. E. and Soldano, B. A., Z. Elektrochem. *57*, 162 (1953).
9. Soldano, B. and Larson, Q. V., J. Am. Chem. Soc. *77*, 1331 (1955).
10. Gregor, H. P., J. Am. Chem. Soc. *73*, 642 (1951).
11. Gregor, H. P., Belle, J., and Marcus, R. A., J. Am. Chem. Soc. *76*, 1984 (1954).
12. Gregor, H. P., Belle, J., and Marcus, R. A., J. Am. Chem. Soc. *77*, 2713 (1955).
13. Gregor, H. P., Hamilton, M. J., Becher, J., and Bernstein, F., J. Phys. Chem. *59*, 874 (1955).
14. Soldano, B., Larson, Q. V., and Myers, G. E., J. Am. Chem. Soc. *77*, 1339 (1955).
15. Soldano, B. and Chesnut, D., J. Am. Chem, Soc. *77*, 1334 (1955).

16. GLUECKAUF, E., Proc. Roy. Soc. *A 214*, 207 (1952).
17. DONNAN, F. G. and GUGGENHEIM, E. A., Z. phys. Chem. *A 162*, 346 (1932).
18. GREGOR, H. P., SUNDHEIM, B. R., HELD, K. M., and WAXMAN, M. H., J. Coll. Sci. *7*, 511 (1952).
19. PEPPER, K. W., J. Appl. Chem. *1*, 124 (1951).
20. SUNDHEIM, B. R., WAXMAN, M. H., and GREGOR, H. P., J. Phys. Chem. *57*, 974 (1953).
21. WAXMAN, M. H., SUNDHEIM, B. R., and GREGOR, H. P., J. Phys. Chem. *57*, 969 (1953).
22. GREGOR, H. P. and FREDERICK, M., Ann. N. Y. Acad. Sci. *57*, 87 (1953).
23. GREGOR, H. P., GUTOFF, F., and BREGMAN, J. I., J. Coll. Sci. *6*, 245 (1951).
24. PEPPER, K. W., REICHENBERG, D., and HALE, D. K., J. Chem. Soc. *1952* 3129 (1952).
25. GREGOR, H. P., HELD, K. M., and BELLIN, J., Anal. Chem. *23*, 620 (1951).
26. DAVIES, C. W. and YEOMAN, G. D., Trans. Farad. Soc. *49*, 968 (1953).
27. DAVIES, C. W. and YEOMAN, G. D., Trans. Farad. Soc. *49*, 975 (1953).

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Zur Kenntnis der Arylborsäuren. I

Von Kurt Torssell

Einleitung

Aromatische Borverbindungen wurden zum erstenmal von Michaelis und Mitarbeitern durch Umsetzung von Diarylquecksilber mit Bortrichlorid oder Bortribromid dargestellt (1)

$$HgAr_2 + BCl_3 \rightarrow ArHgCl + ArBCl_2 \xrightarrow{\text{Hydrolyse}} ArB(OH)_2 \quad (a)$$

Nach der Entdeckung der magnesiumorganischen Verbindungen und ihrer Reaktionen wurden die Arylborsäuren (a) leichter zugänglich. Arylmagnesiumhalogenid setzt sich mit Bortrifluorid (2) oder besser Tributylborat (3) unter Bildung von Arylborsäure um.

$$\left.\begin{matrix} BF_3 \\ B(OBu)_3 \end{matrix}\right\rangle \xrightarrow[2)\ \text{Hydrolyse}]{1)\ ArMgX} ArB(OH)_2$$

Auf diese Weise lassen sich in oft guter Ausbeute alkyl-, alkoxy- und halogensubstituierte Phenylborsäurederivate darstellen. Die Methode erlaubt aber nicht eine unmittelbare Darstellung bororganischer Verbindungen, die andere reaktive funktionelle Gruppen, wie die Amino-, Nitro-, Carboxy-, Formyl-, Sulfogruppen usw., enthalten. Man ist deshalb darauf angewiesen, solche Gruppen einzuführen, nachdem das Bor mit Kohlenstoff im Molekül verknüpft worden ist. Bei einem solchen Verfahren besteht aber immer die Gefahr, dass die Dihydroxyborgruppe während der Umsetzung oder Umlagerung abgespalten wird. Die Stabilität der B–C-Bindung verschiedener Verbindungen variiert stark. Es gibt Arylborsäurederivate, die von selbst langsam an der Luft zerfallen, z. B. *p*-Amino-phenylborsäure XV. 2-Nitro-4-carboxy-phenylborsäure II dagegen wurde von rauchender Salpetersäure bei 80° (5 Min.) nicht angegriffen. Beim Eingiessen der Reaktionsmischung in Eiswasser wurde das Ausgangsprodukt unverändert zurückgewonnen. Im Laufe der Arbeit hat es sich herausgestellt, dass sich die erwünschten Substitutionsprodukte oft durch Wählen geeigneter Synthesewege und Versuchsbedingungen gewinnen lassen.

Diese Arbeit wurde aus zwei Gesichtspunkten begonnen. Neben dem präparativ-organischen Interesse dieser Verbindungen ist es denkbar, dass sie physiologische Wirkungen besitzen können. Zur Frage der physiologischen Effekte wird auf die Teile V und VI hingewiesen. Es soll nur angedeutet werden, dass besonders *p*-aminosubstituierte Derivate in diesem Zusammenhang von Interesse sind.

Besprechung der Versuche

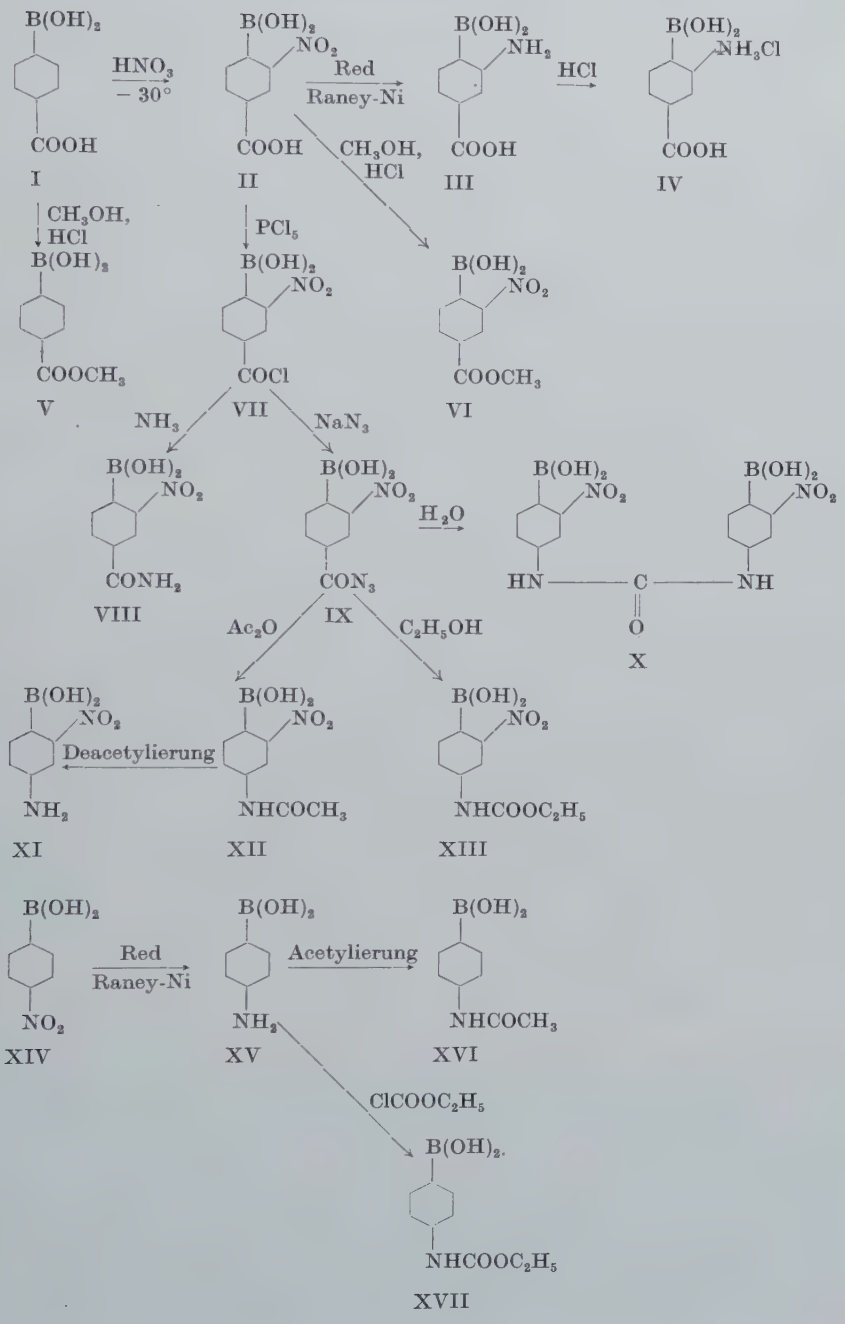
Es wurde zunächst versucht, die einfache *p*-Amino-phenylborsäure darzustellen. Der einfachste Weg wäre natürlich, die leicht zugängliche Phenylborsäure zu nitrieren und dann das *p*-Nitroderivat XIV zum Amin zu reduzieren. Beim Nitrieren bildet sich jedoch das *p*-Derivat XIV in einem zum *o*-Derivat ungünstigen Mengenverhältnis. Es lässt sich nur in einer Ausbeute von höchstens 10% (4, 5) gewinnen. Deshalb wurde im Anfang auf diese Methode verzichtet. Es wurde zunächst versucht, *p*-Carboxy-phenylborsäure I nach Schmidt abzubauen. Sie reagiert sehr träge und wegen der Schwerlöslichkeit der Borverbindung in den bei dieser Synthese gebräuchlichen Lösungsmitteln was es auch schwierig, sie zur Reaktion zu bringen. Nach mehrstündigem Behandeln wurde die Ausgangssubstanz I zum grössten Teil zurückgewonnen. Wenig Anilin wurde nachgewiesen. Hofmannscher Abbau konnte auch nicht verwendet werden, da unsere Versuche, das Carbonsäureamid darzustellen, scheiterten. *p*-Carboxy-phenylborsäure I konnte eigentümlicherweise weder mit Thionylchlorid noch mit Phosphorpentachlorid zur Reaktion gebracht werden. Es ist bemerkenswert, dass die B–C-Bindung dabei trotz des Erhitzens auf etwa 100° nicht angegriffen wurde. *p*-Carbäthoxy- und *p*-Carbomethoxy-phenylborsäure V wurden nach zweitägigem Stehen in gesättigter ammoniakalischer Äthanollösung unverändert zurückgewonnen, so dass das erwünschte Carbonsäureamid auch auf diesem Wege nicht erhalten werden konnte. *m*-Carboxy-phenylborsäure verhält sich in ähnlicher Weise.

Die erste Synthesemethode wurde wieder aufgenommen; das *p*-Nitroderivat XIV liess sich leicht mit Raney-Nickel katalytisch hydrieren. Das Amin, aus Wasser umkristallisiert, bildete weisse Nadeln, die sich aber langsam an der Luft unter Bildung von Anilin und Borsäure zersetzten. Das Acetylderivat XVI ist stabil.

Als Ausgangssubstanz für die folgenden Umsetzungen diente *p*-Carboxy-phenylborsäure I. Nach früheren Angaben über die Nitrierung von Phenylborsäure und *p*-Tolylborsäure ist es wichtig, die Temperatur möglichst niedrig zu halten, da sonst die Gefahr besteht, dass die Dihydroxyborgruppe abgespalten wird (5, 6, 7). Es wurde deshalb versucht, *p*-Carboxy-phenylborsäure I mit rauchender Salpetersäure (Dichte 1,50) bei $-30°$ zu nitrieren. 2-Nitro-4-carboxy-phenylborsäure II wurde dabei in 60 bis 70%iger Ausbeute gewonnen. Es erwies sich aber später, dass *p*-Carboxy-phenylborsäure und 2-Nitro-4-carboxy-phenylborsäure II so stabile Verbindungen sind, dass die Nitrierung ohne Gefahr bei Zimmertemperatur durchgeführt werden kann. Da sowohl die Carboxygruppe als auch die Dihydroxyborgruppe in *meta*-Stellung dirigieren, ist zu erwarten, dass auch 3-Nitro-4-carboxy-phenylborsäure gebildet wird. Die isomere Verbindung konnte aber nicht nachgewiesen werden, was auf ihre Leichtlöslichkeit in Wasser zurückzuführen ist (siehe Teil II). Die *meta*-dirigierende Wirkung der Carboxygruppe ist allerdings stärker als die der Dihydroxyborgruppe.

2-Nitro-4-carboxy-phenylborsäure II setzte sich in gewöhnlicher Weise mit Phosphorpentachlorid unter Bildung von Carbonsäurechlorid VII um, das mit Ammoniak das entsprechende Amid VIII und mit Natriumazid das Carbonsäureazid IX bildete. Beim Erwärmen in Essigsäureanhydrid, mit etwas Phosphorsäure versetzt, lagerte sich das Azid nach Curtius um und das acetylierte Amin XII wurde in guter Ausbeute erhalten. In reinem Essigsäureanhydrid oder in Essigsäureanhydrid mit etwas Schwefelsäure anstatt Phosphorsäure versetzt, trat erhebliche Zersetzung ein, wobei *m*-Nitro-acetanilid erhalten wurde. Es ist jedoch bemerkenswert,

Reaktionsschema



dass die Borverbindung in siedendem mit Phosphorsäure angesäuertem Essigsäureanhydrid stabil ist. Vorsichtige Deacetylierung ergab das freie Amin. Auch andere Curtiussche Umlagerungen wurden durchgefürt. Mit Äthanol bildete sich der Carbaminsäureäthylester XIII und mit Wasser der entsprechende Harnstoff.

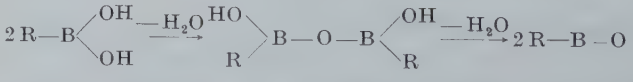
Die Carboxylgruppe der *p*-Carboxy- und 2-Nitro-4-carboxy-phenylborsäure liess sich mit salzsaurem Methyl- und Äthylalkohol leicht zum entsprechenden Ester umwandeln. Um das Carbonsäurehydrazid zu gewinnen, wurde in einem Versuch 2-Nitro-4-carbomethoxy-phenylborsäure VI mit Hydrazinhydrat in siedendem Methanol umgesetzt. Bei dieser Behandlung wurde aber die Dihydroxyborgruppe abgespalten, *m*-Nitrobenzhydrazid konnte in 60%iger Ausbeute isoliert werden.

Die Nitrogruppe der 2-Nitro-4-carboxy-phenylborsäure II liess sich leicht katalytisch hydrieren. Das Amin III ist in Wasser und den gebräuchlichsten Lösungsmitteln schwerlöslich. Sein Hydrochlorid IV lässt sich aus verdünnter Salzsäure umkristallisieren; im Wasser bildet sich das freie Amin zurück. Es wurde auch versucht 2-Nitro-4-amino-phenylborsäure XI zu hydrieren. Das Amin ist aber sehr unbeständig und spaltet Borsäure ab.

Unsere Versuche, 2-Amino-4-carboxy-phenylborsäure III zu diazotieren und zu Phenol umzuwandeln sind gescheitert. Zwar bildet sich das Diazoniumsalz (mit β-Naphtol nachgewiesen), nach Erwärmen der Lösung aber konnte nur wenig von einer Substanz, die kein Bor enthielt, isoliert werden.

Die Arylborsäuren zeichnen sich durch ihre Kristallisationsfreudigkeit aus. Sie sind gewöhnlich in heissem Wasser ziemlich leicht löslich und weniger in kaltem, weshalb Wasser zum Umkristallisieren sehr geeignet ist. Polare organische Lösungsmittel wie Aceton, Äthylacetat, Acetonitril und Nitromethan sind gute Lösungsmittel für die Arylborsäuren, während die unpolaren wie Benzol, Tetrachlorkohlenstoff schlechter lösen. In Petroläther sind sie fast unlöslich, was natürlich mit ihrem starken polaren Charakter zusammenhängt. In manchen Fällen lassen sie sich besonders aus Nitromethan schön umkristallisieren. In Methyl- und Äthylalkohol sind alle Arylborsäuren leicht löslich, was auf einer Veresterung der Dihydroxyborgruppe beruht. Die Ester der einfachsten Arylborsäuren sind Flüssigkeiten, die sich beim Zusatz von Wasser sofort hydrolysieren (8).

Es erwies sich als schwierig, die Schmelzpunkte und besonders die Zersetzungspunkte, um die es sich meistens handelte, zu reproduzieren. Die Dihydroxyborgruppe verliert beim Erhitzen Wasser und geht allmählich in ihre halbanhydrische oder anhydrische Form über:



Dieses Verhalten verursacht ein mehr oder weniger grosses Schmelzpunktsintervall, das von der Geschwindigkeit des Erhitzens abhängig ist. Um dies möglichst zu verhindern, wurde der Schmeltzpunktsapparat bei der Bestimmung auf eine Temperatur vorgewärmt, die etwa 20° niedriger als der Schmelzpunkt war.

Die Arylborsäuren haben fast durchgehend einen hohen Schmelzpunkt bzw. Zersetzungspunkt — zwischen 200 und 300°. Die Angaben sind korrigiert worden.

Eine wässrige Silbernitratlösung spaltet in glatter Reaktion die Dihydroxyborgruppe ab, wobei diese gegen Wasserstoff ausgetauscht wird (7). Es ist denkbar, dass als Zwischenprodukt bei dieser Reaktion eine silberorganische Verbindung auftritt, die aber sofort in Kohlenwasserstoff und Silberhydroxyd zerfällt.

Wenn man ein Phenylborsäurederivat noch weiter substituiert, z.B. durch Nitrierung, lässt sich die Lage der neu eingetretenen Substituenten durch Abspalten der Dihydroxyborgruppe und Identifizieren des Benzolderivates bestimmen. Siehe den Strukturbeweis der 2-Nitro-4-carboxy-phenylborsäure.

$$C_6H_5—B(OH)_2 \xrightarrow[H_2O]{Ag^+} C_6H_6 + H_3BO_3$$

Die Arylborsäuren setzen sich mit Sublimat nach folgender Gleichung um, wobei eine Merkurierung eintritt:

$$ArB(OH)_2 + HgCl_2 \xrightarrow{H_2O} ArHgCl + H_3BO_3 + HCl$$

Die Reaktion eignet sich teils zur quantitativen Bestimmung des Bors (9), teils zum Strukturbeweis. *Es soll in diesem Zusammenhang auf die präparative Bedeutung der Reaktion hingewiesen werden, die durch die Zugänglichkeit der Arylborsäuren einen neuen Weg zur Gewinnung quecksilberorganischer Verbindungen bietet.* Die Reaktion scheint um so rascher zu gehen, je negativer geladen das bortragende Kohlenstoffatom ist. Betreffs des Reaktionsmechanismus wird auf die vierte Mitteilung dieser Serie hingewiesen.

Experimenteller Teil

4-Carboxy-phenylborsäure I. Diese Säure wurde aus *p*-Methyl-phenylborsäure durch Oxydation mit Kaliumpermanganat in alkalischer Lösung nach Michaelis (10) dargestellt. Ausbeute: 76%.

p-Carbomethoxy-phenylborsäure V. 0,45 g *p*-Carboxy-phenylborsäure I wurden mit 4 ml Methylalkohol, der 10% HCl enthielt, eine Stunde lang auf 60° erwärmt. Die Lösung wurde in 15 ml Eiswasser gegossen, wobei ein weisser Niederschlag entstand, der sich aus Wasser umkristallisieren liess. Ausbeute: 0,34 g oder 77%. Schmp. 230–3°. $C_8H_7O_3B$ (162,0, Anhydrid): Ber. C 59,3; H 4,36; gef. C 59,0; H 4,65.

Der *Äthylester* lässt sich analog darstellen. Er sintert bei 135° und schmilzt bei etwa 160°. Kuivila *et al.* geben den Schmp. 198–200° an (11). Die Substanz wurde aus Nitromethan umkristallisiert. Weisse, körnige Kristalle. Ausbeute: 81%. $C_9H_{11}O_4B$ (194,0): Ber. C 55,7; H 5,72; gef. C 55,4; H 5,50.

Die Versuche, die Methyl- oder Äthylester mit alkoholischem oder wässrigem Ammoniak in das entsprechende *Carbonsäureamid* zu überführen, waren erfolglos. Es konnte auch nach 4-stündigem Kochen unter Rückfluss mit Hydrazinhydrat in wenig Alkohol kein *Carbonsäurehydrazid* nachgewiesen werden.

2-Nitro-4-carboxy-phenylborsäure II. Zu 60 ml rauchender Salpetersäure (hellgelb), Dichte 1,50, die auf $-25°$ bis $-30°$ abgekühlt war, wurden unter Umrühren 10 g gepulverte *p*-Carboxy-phenylborsäure I portionsweise zugesetzt. Nach dem Zusatz wurde noch eine Stunde bei derselben Temperatur gerührt, wobei die Säure allmählich vollständig in Lösung ging. Die Mischung wurde auf 200 ml Eiswasser gegossen und der gebildete Niederschlag nach zweistündigem Stehen in der Kälte abfiltriert und mit Eiswasser gewaschen. Aus Wasser–Äthanol (3 : 1) umkristallisiert, zersetzt sich die Substanz bei 250° bis 260°, je nach der Geschwindigkeit des Erhitzens. Blassgelbe Nadeln. Es konnte kein Isomer nachgewiesen werden. Ausbeute: 8,5 g oder 67%. Leicht löslich in Methanol und Äthanol. Wenig löslich in

kaltem Wasser, besser in heissem Wasser. Schwerlöslich in Benzol. $C_7H_6O_6NB$ (211,0): Ber. C 39,9; H 2,87; gef. C 39,9; H 2,85.

Konstitutionsbeweis. 0,4 g der Nitroverbindung II wurden in einem kleinen E-Kolben mit 10 ml 0,5 *M* $AgNO_3$-Lösung 10 Min. vorsichtig gekocht. Beim Kühlen schieden sich weisse Nadeln ab. Ein Teil wurde aus Wasser umkristallisiert. Zersp. 311°. (Das Ag-Salz der *m*-Nitro-benzoesäure.) Der Rest wurde in wenig heissem mit etwas Schwefelsäure schwach angesäuertem Wasser gelöst und mit verdünnter Salzsäure versetzt, wobei Silberchlorid ausfiel. Die Lösung wurde heiss filtriert und beim Kühlen kamen weisse Kristalle heraus, die, aus Wasser umkristallisiert, den Schmelzpunkt und Mischschmelzpunkt 141–142° hatten. *m*-Nitro-benzoesäure schmilzt bei 141,4°. Ausbeute: 60%.

2-Nitro-4-carbomethoxy-phenylborsäure VI. Man liess eine Lösung von 0,35 g 2-Nitro-4-carboxy-phenylborsäure II in 2 ml Methanol (10% HCl-Gehalt) eine Stunde bei 60° stehen. Die Mischung wurde dann in 10 ml Eiswasser gegossen, wobei der Ester ausfiel. Nach Stehen in der Kälte wurde der Niederschlag abfiltriert und bei 60° getrocknet. Aus Nitromethan oder Wasser umkristallisiert, fängt die Substanz bei 160° zu sintern an und schmilzt bei 168°. Körnige, leicht gelbliche Kristalle. Ausbeute: 0,32 g oder 86%. Leicht löslich in Alkohol. $C_8H_8O_6NB$: Ber. C 42,7; H 3,58; gef. C 42,4; H 3,47.

Analog entsteht der *Äthylester*. Er lässt sich aus Nitromethan oder Wasser umkristallisieren. Körnige, leicht gelbliche Kristalle, die bei 115° sintern und unter Gasentwicklung bei 125° schmelzen. Ausbeute: 90%. Die Substanz enthält ein Mol Kristallwasser. $C_9H_{10}O_6NB.H_2O$ (257,0): Ber. C 42,1; H 4,71; gef. C 42,1; H 4,76.

Umsetzungen von Methyl- und Äthylester mit Hydrazinhydrat. Der Methylester VI wurde in wenig Methanol mit Hydrazinhydrat (Molverhältnis 1 : 1,1) sechs Stunden unter Rückfluss gekocht. In 60%iger Ausbeute wurde das *m*-Nitro-benzhydrazid isoliert. Schmelzpunkt und Mischschmelzpunkt: 151–152°. Die Dioxyborgruppe wurde also bei dieser Behandlung abgespalten. Die Reaktion kann auch als Konstitutionsbeweis dienen. Der Äthylester reagiert in derselben Weise. In einem anderen Versuch wurde nur 1,5 Stunden unter Rückfluss gekocht, wobei der Äthylester in 47%iger Ausbeute das *m*-Nitro-äthylbenzoat, und in 23%iger Ausbeute das *m*-Nitro-benzhydrazid lieferte. Die Phenylborsäure selbst ist gegen Hydrazinhydrat stabil.

2-Amino-4-carboxy-phenylborsäure III. 2,0 g 2-Nitro-4-carboxy-phenylborsäure II in 20 ml Methanol gelöst wurden bei Atmosphärendruck mit Raney-Nickel katalytisch hydriert. Nach sechs Stunden war die theoretische Menge von Wasserstoff aufgenommen. Das Raney-Nickel wurde abfiltriert und das Filtrat mit 75 ml Wasser versetzt, wobei ein gelber Niederschlag ausfiel. Die Substanz wurde abfiltriert, mit Wasser gewaschen und im Exiccator getrocknet. Die alkoholische Lösung wurde beim Stehen an der Luft allmählich dunkel. Ausbeute: 89%. Zersetzt sich bei 180–190°. Schwerlöslich in Wasser, besser in Alkohol und Eisessig. $C_7H_8O_4NB$ (180,9): Ber. C 46,4; H 4,42; gef. C 46,5; H 4,35.

Das *HCl-Salz des Amins* IV wurde folgendermassen dargestellt: 2,5 g 2-Nitro-4-carboxy-phenylborsäure II wurden wie oben katalytisch hydriert. Zu der alkoholischen Lösung wurden 5 ml konz. Salzsäure zugesetzt, wobei nach kurzer Zeit gelbe Nadeln des Hydrochlorids ausfielen. Das Salz wurde aus verdünnter Salzsäure umkristallisiert. Beim Umkristallisieren aus Wasser wurde das Amin zurückgebildet. Ausbeute: 1,9 g oder 74%. Zersetzt sich unter Gasentwicklung bei etwa 270°. $C_7H_9O_4NClB$ (217,4): Ber. Cl 16,31; gef. Cl 16,26.

Analog wird das *Sulfat* dargestellt.

Diazotierungsversuche. Unsere Versuche, 2-Amino-4-carboxy-phenylborsäure III zu diazotieren und in das entsprechende Hydroxyderivat zu überführen waren erfolglos. In einem Versuch wurde etwas Hydrochlorid in verdünnter Salzsäure aufgelöst. Beim Zusatz der Nitritlösung trat Gasentwicklung ein und beim Aufarbeiten wurde eine Substanz erhalten, die kein Bor enthielt, weshalb auf weitere Versuche verzichtet wurde.

2-Nitro-phenylborsäure-4-carbonsäurechlorid VII. 3,2 g (15 mMol) fein gepulverte 2-Nitro-4-carboxy-phenylborsäure II wurden mit 6,3 g (30 mMol) fein gepulvertem Phosphorpentachlorid in einem Kolben mit Rückflusskühler innig gemischt und auf ein Wasserbad bei 60–70° gesetzt. Eine lebhafte Reaktion setzte ein, die nach etwa einer Stunde beendet war. Das Reaktionsgemisch war völlig verflüssigt. Das Phosphoroxychlorid wurde im Vakuum abdestilliert. Im Kolben verblieb eine hellgelbe feste Substanz, die aus Säurechlorid bestand. Sie war genügend rein, um für weitere Synthesen verwendet werden zu können. Man muss die doppelte berechnete Menge von Phosphorpentachlorid zusetzen, weil die vorhandene Dioxyborgruppe Wasser abspaltet und in die anhydrische Form übergeht. Thionylchlorid greift die Carboxylgruppe nicht an. Das Säurechlorid ist in Aceton leicht löslich. Ausbeute fast quantitativ.

2-Nitro-phenylborsäure-4-carbonsäureamid VIII. Das Säurechlorid, aus 1,1 g 2-Nitro-4-carboxy-phenylborsäure und 2,2 g Phosphorpentachlorid dargestellt (siehe oben), wurde portionsweise unter Kühlung mit Eis zu 4 ml konz. NH_3 zugesetzt. Die Lösung wurde dann mit verdünnter Salzsäure schwach angesäuert und nach Stehen über Nacht in der Kälte kamen hellgelbe Kristalle heraus, die, aus Wasser umkristallisiert, den Zersetzungspunkt 185–187° hatten. Leicht löslich in Äthanol. Löslich in Wasser. Ausbeute: 0,72 g oder 66%. $C_7H_7O_5N_2B$ (210,0): Ber. C 40,0; H 3,36; gef. C 40,3; H 3,36.

2-Nitro-phenylborsäure-4-carbonsäureazid IX. Das Säurechlorid, aus 3,2 g 2-Nitro-4-carboxy-phenylborsäure II dargestellt, wurde in 10 ml Aceton gelöst. Unter kräftigem Schütteln und Kühlung in einem Eisbad wurden 1,15 g Natriumazid, gelöst in 3 ml Wasser, tropfenweise zugegeben. Man liess die gebildete Suspension 10 Min. stehen, filtrierte ab und wusch mit etwas Aceton nach. Das Filtrat wurde mit 60 ml Wasser versetzt. Nach Stehen über Nacht in der Kälte fiel das Azid als blassgelbe Nadeln aus. Sie wurden abfiltriert, mit Wasser gewaschen und im Exsiccator getrocknet. Die Substanz zersetzt sich unter Gasentwicklung bei etwa 100–110°. Sie verpufft in der Flamme. Wegen der Zersetzlichkeit der Substanz wurde auf eine Analyse verzichtet. Die folgenden Umsetzungen zeigen, dass das Azid vorhanden ist. Ausbeute: 2,7 g oder 76 %.

2-Nitro-4-carbäthoxyamino-phenylborsäure XIII. 0,5 g 2-Nitro-phenylborsäure-4-carbonsäureazid wurden vorsichtig mit 4 ml Äthanol eine halbe Stunde unter Rückfluss gekocht. Es trat sofort Gasentwicklung ein und die Lösung färbte sich gelb. 10 ml Wasser wurden zugegeben, wobei nach kurzer Zeit ein gelber Niederschlag ausfiel. Er wurde abfiltriert, mit Wasser gewaschen und im Exsiccator getrocknet. Rohausbeute: 0,41 g. Lässt sich aus wenig Nitromethan umkristallisieren. Blassgelbe Nadeln, die sich bei 113–114° unter Aufschäumen zersetzen. $C_9H_{11}O_6N_2B$ (254,0): Ber. C 42,6; H 4,37; gef. C 42,4; H 4,56.

Das *Benzylurethan* entsteht analog. 0,35 g Azid IX wurden mit 0,20 g Benzylalkohol in 2 ml Toluol 20 Min. unter Rückfluss gekocht. Es bildete sich ein Niederschlag, der nach Erkalten abfiltriert wurde. Er liess sich aus Wasser–Äthanol (3 : 1)

umkristallisieren. Die aus der Lösung zuerst ausgefallenen Öltröpfchen wandeln sich bald in leicht gelbliche Nadeln um. Zersp. 145°. Ausbeute: 0,3 g. $C_{14}H_{13}O_6N_2B$ (316,1): Ber. C 53,2; H 4,15; gef. C 53,1; H 4,33.

N,N'-bis-[3-nitro-phenylborsäure-(4)*]-harnstoff* X. 1,0 g 2-Nitro-phenylborsäure-4-carbonsäureazid IX wurde eine Stunde in einer Mischung von 5 ml Aceton und 5 ml Wasser unter Rückfluss gekocht. Es setzte sofort Gasentwicklung ein und allmählich kamen gelbe, körnige Kristalle heraus, die nach Erkalten abfiltriert und mit Wasser gewaschen wurden. Schwerlöslich in Wasser und Nitromethan; besser in Alkohol. Die Substanz wurde durch Auflösen in Methanol und Ausfällen mit Wasser gereinigt. Ausbeute 51%. Zersp. 220–230°, je nach der Geschwindigkeit des Erhitzens. $C_{13}H_{12}O_9N_4B_2$ (389,9): Ber. C 40,0; H 3,11; gef. C 40,2; H 3,16.

2-Nitro-4-acetylamino-phenylborsäure XII. In einem 50-ml-Kolben wurden zu 3 g gepulverter 2-Nitro-phenylborsäure-4-carbonsäureazid in 20 ml Essigsäureanhydrid eine Mischung von 1,5 ml Phosphorsäure (Dichte 1,75) und 5 ml Essigsäureanhydrid gegeben. Es trat sofort Wärmeentwicklung ein und das Azid löste sich vollständig auf. Die Lösung erstarrt aber beim Stehen zu einer Gallerte. Um dies möglichst zu verhindern, wurde sie gut umgerührt. Die Mischung wurde auf einem Schwefelsäurebad auf 100° bis 110° drei Viertelstunden erhitzt, danach wurde sie in 130 ml Eiswasser gegossen. Es fielen blassgelbe sternförmige Kristalle aus, die nach Stehen in der Kälte abfiltriert und mit Eiswasser gewaschen wurden. Rohausbeute: 2,0 g oder 70%. Rohschmp. 150–155°. Nach mehrmaligem Umkristallisieren aus Wasser schmolz die Substanz bei 171–173° (Zersetzungspunkt). Sie lässt sich auch aus Nitromethan umkristallisieren. $C_8H_9O_5N_2B$ (224,0): Ber. C 42,8; H 4,05; N 12,50. Gef. C 42,2; H 3,97; N 12,82.

2-Nitro-4-amino-phenylborsäure XI. 0,85 g von der entsprechenden N-Acetylverbindung XII wurden mit 15 ml 5 *N* Salzsäure sieben Minuten gekocht. Die Lösung wurde etwas abgekühlt und dann warm mit 5 *N* Natriumhydroxyd bis zur schwach sauren Reaktion neutralisiert. Man erkennt leicht den Endpunkt, denn die Lösung nimmt dabei eine rötliche Farbe an. Die Lösung wurde schnell heiss filtriert. Aus dem Filtrat kamen beim Erkalten gelbe Stäbchen des Amins heraus. Sie lassen sich aus Wasser oder, besser, aus Nitromethan–Acetonitril (2 : 1) umkristallisieren. Zersp. 142–143°. Ausbeute: 0,54 g oder 78%. Nach der Stickstoffanalyse ist die Substanz halb anhydrisch. $C_{12}H_{12}O_7N_4B_2$, $[NH_2(NO_2)C_6H_3BOH]_2O$ (345,9): Ber. N 16,20; gef. N 16,07.

Es gelang nicht, *2,4-Diamino-phenylborsäure* darzustellen. 0,58 g 2-Nitro-4-amino-phenylborsäure wurden in 15 ml abs. Äthanol mit Raney-Nickel katalytisch hydriert. Die Reaktion war in einer halben Stunde beendet. Das Raney-Nickel wurde abfiltriert und die alkoholische Lösung unter Stickstoff im Vakuum eingedampft. An der Luft färbte sie sich bald rotbraun. Im Kolben blieb eine dickflüssige Flüssigkeit zurück. Ein ml Wasser wurde hinzugefügt, wonach die Destillation fortgesetzt wurde. Zu dem Rückstand wurden 3 ml Aceton zugesetzt, wobei sich ein weisser Niederschlag bildete, der sich als Borsäure erwies. Das Amin ist unbeständig und spaltet leicht Borsäure ab.

p-Acetylamino-phenylborsäure XVI. 0,5 g *p*-Nitro-phenylborsäure (5) in 15 ml absol. Alkohol wurden bei Zimmertemperatur und Atmosphärendruck mit Raney-Nickel hydriert. Die alkoholische Lösung wurde im Vakuum unter Stickstoff fast zur Trockne eingeengt. 2 ml Wasser und 1 ml Essigsäureanhydrid wurden zugefügt. Es wurde geschüttelt und eine Viertelstunde auf 50 bis 60° erwärmt. Beim Erkalten fielen weisse blätterförmige Kristalle aus vom Rohschmp. 225–226°, 0,21 g oder 39%.

Sie wurden aus wenig Wasser umkristallisiert. Schmp. 230–231°. Gef. C 54,32; H 5,41; ber. für $C_8H_{10}O_3NB$ (179,0) C 53,68; H 5,63.

p-Amino-phenylborsäure XV. Wie oben beschrieben, wurden 0,5 g *p*-Nitro-phenylborsäure XIV katalytisch hydriert. Die Alkohollösung wurde nach Zusatz von etwas Wasser zur Trockne eingeengt und der Rückstand möglichst unter Stickstoff aus wenig Wasser umkristallisiert. Weisse Nadeln, die sich an der Luft langsam unter Bildung von Anilin und Borsäure zersetzen. Das acetylierte Amin ist stabil. Ausbeute: 0,2 g.

Es wurde auch versucht, *p*-Carboxy-phenylborsäure nach Schmidt umzulagern. Sie reagiert sehr träge und wegen der Schwerlöslichkeit der Borverbindung war es schwierig, sie zur Reaktion zu bringen. Nach mehrstündigem Behandeln wurde die *p*-Carboxy-phenylborsäure in guter Ausbeute zurückgewonnen. Wenig Anilin wurde nachgewiesen.

p-Carbäthoxyamino-phenylborsäure XVII. Die Mutterlauge von der Umkristallisation des Amins wurde mit etwas Natriumcarbonat und Chlorameisensäureäthylester unter Kühlung geschüttelt. Der Carbaminsäureäthylester fiel sofort aus und wurde aus Nitromethan umkristallisiert. Weisse Nadeln vom Schmp. 221–222°. Gef. N 6,91; ber. für $C_{18}H_{22}O_7N_2B_2$ (halbanhydrisch, 400,0) N 7,00.

Zusammenfassung

Die Darstellung und Eigenschaften einiger neuer Arylborsäuren sind beschrieben worden. Die Bor–Kohlenstoff-Bindung erwies sich unerwartet stabil gegen viele chemische Reagenzien. Das hat eine Reihe von Umsetzungen ermöglicht, die nicht vorausgesehen werden konnten. Nitrierung, Hydrierung, Curtiussche Reaktion sind z. B. unter scharfen Bedingungen durchgeführt worden, ohne dass die Dihydroxyborgruppe abgespalten wird. Es ist beabsichtigt, aus den allgemeinen Eigenschaften der in dieser und in den folgenden Mitteilungen dargestellten Arylborsäuren, Schlüsse über Stabilität und Verhalten der B–C-Bindung zu ziehen. Einige Derivate sind besonders im Hinblick auf sterische Ähnlichkeiten mit physiologisch wirksamen Substanzen dargestellt.

Herrn Prof. Dr. KARL MYRBÄCK, dem Direktor des Instituts für organische Chemie und Biochemie, sage ich für seine wertvollen Anregungen und sein liebenswürdiges Interesse für diese Arbeit meinen ergebensten Dank.

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LITERATUR

1. MICHAELIS, A. und BECKER, P., *Ber. 13* (1880), 58; *15* (1882), 180.
2. KRAUSE, E. und NITSCHE, R., *Ber. 55* (1922), 1261.
3. KHOTINSKY, E. und MELAMED, M., *Ber. 42* (1909), 3090.
4. BETTMAN, B., BRANCH, G. E. K. und YABROFF, D. L., *J. Am. Chem. Soc. 56* (1934), 1865.
5. SEAMAN, W. and JOHNSON, J. R., *J. Am. Chem. Soc. 53* (1931), 711.
6. AINLEY, A. D. und CHALLENGER, F., *J. Chem. Soc. 1930*, 2171.
7. BEAN, F. R. und JOHNSON, J. R., *J. Am. Chem. Soc. 54* (1932), 4415.
8. TORSSELL, K., *Acta Chem. Scand. 8* (1954), 1779.
9. WITTIG, G., KEICHER, G., RÜCKERT, A. und RAFF, P., *Ann. 563* (1949), 110.
10. MICHAELIS, A. und RICHTER, E., *Ann 315* (1901), 29.
11. KUIVILA, H. G. und HENDRICKSON, A. R., *J. Am. Chem. Soc. 74* (1952), 5068.

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Improved method for the analysis of pyridine bases

By Gösta Lindner

With 1 figure in the text

In this paper a number of methods used for manufacture of pyridine bases from raw pyridine from gasworks are discussed. For small scale manufacture a method working with dry HCl-gas has been chosen as one, that gives rather pure picolines, lutidines and collidines in a simple way, avoiding a great many corrosion problems, which are disturbing in other methods.

To secure good results a rather fast and reliable method of analysis is necessary and for that reason a number of analytical methods reported in literature are discussed. "Chromatography" on Al_2O_3 impregnated with $CuCl_2$, has been chosen as the most promising method. This method, first published by Vignes *et al.* (1), has been modified and improved. It is shown that the separation of the bases is really not depending on absorption but on the formation of addition compounds between the bases and $CuCl_2$. In the course of the work 20 addition compounds not previously described, between $CuCl_2$ and pyridine bases have been prepared and their compositions confirmed by analysis. Some of them are rather unstable.

During recent years constituents of the so called raw pyridine, apart from pyridine itself and β-picoline, have become of great interest. For instance, the discovery of the antitubercular effect of *iso*nicotinic acid hydrazide greatly increased the importance of the manufacture of γ-picoline. Later on, the price of this compound has become so low owing to new synthetic methods based on citric acid, that production from γ-picoline seems to be too expensive. However, the interest in preparing pure pyridine bases is still so great, that it undoubtedly gives cause for a great deal of work in this field of chemistry.

At the gasworks of Stockholm the raw pyridine is produced both from the condensed water and from the raw benzene. Owing to the high temperature-coking method used there in order to produce a good coke, the raw pyridine has a composition somewhat different from that of other sources. Eguchi (2), Benzie *et al.* (3), Fushizaki (4) and Dodonow *et al.* (5), have examined raw pyridine from shale oil, Hackmann *et al.* (6), Bratton *et al.* (7), Mahan *et al.* (8) and Densham *et al.* (9) raw pyridine from petrol. Every possible pyridine homologue with methyl- and ethyl-substituents with the exception of 3,4,5-collidine has been identified by fractionated distillation followed by crystallization of compounds with, e.g., $HgCl_2$, $ZnCl_2$, picric acid, styphnic acid and picrolonic acid. Raw pyridine from gasworks has been examined especially in Germany and the results are reported in many articles, which are comprehensively summarized by Winkler (10). The only difference is that the ethyl-substituted pyridines are more scarce here.

Raw pyridine produced at cokeworks is, as we have found, almost lacking in this type of homologues and only the normal methyl-substituted picolines, lutidines and collidines can be identified. This is, by the way, in accordance with the fact that ethyl benzene is lacking in the raw benzene produced at cokeworks.

Manufacture of pure pyridine bases

Pure pyridine and α-picoline can be manufactured by fractionated distillation in effective columns owing to the large difference in boiling points. As to the so called β-fraction with the constituents β-picoline, γ-picoline, and 2,6-lutidine which, without being an azeotrope, cannot be separated by distillation, a great many separation methods have been reported in literature, especially owing to the great importance of nicotinic acid. Double salts with, e.g., $ZnCl_2$, $HgCl_2$, picric acid, and salts with oxalic, benzoic, phthalic and phosphoric acids, obtained with or without inoculation, are described by, among others, Lidstone (11), Engel (12), Slagle *et al.* (13) and Cracas (14). These methods are time-consuming and do not give pure products. Owing to the larger differences in boiling points the hydrohalogenides of the bases are separable by distillation (Cislak *et al.*, 15) but the distillation is difficult to carry out because of the corrosion. Azeotropic distillations were used by Riethof (16) and Coulson (17) with phenol, *o*-chlorophenol, formic acid and other carboxylic acids with 2–5 C atoms. The distillations were performed in rather effective columns but the results were not very satisfying.

Engel (18) has shown that the β-fraction can be treated with either concentrated hydrochloric acid or hydrogen chloride gas in a quantity equivalent to the proportion of the expected homologue in the raw product. 2,6-lutidine will be the first base to crystallize as hydrochloride and if the procedure is repeated the γ-picoline hydrochloride will separate. Pure β-picoline can be manufactured from the residue. The separated hydrohalogenides of the bases can be washed with alifatic, alicyclic and aromatic hydrocarbons and the bases are regenerated with sodium hydroxide. The precipitation can be repeated or the hydrohalogenides recrystallized to obtain very pure products.

A great many methods are concerned especially with the purification of β-picoline and are based on the destruction of 2,6-lutidine and γ-picoline. They make use of the reactivity of methyl groups substituted in α- or γ-position in pyridine in contrast to the relative indifference of methyl groups in β-position. These methods are of no interest here, except one according to Zavody *et al.* (19), which is of special interest because of its extraordinarily good results in manufacturing pure β-picoline. The β-fraction or the earlier mentioned β-picoline residue is treated with either a concentrated solution of $CuCl_2$,aq or with Cu_2Cl_2 at the boiling point of the β-fraction. A red-brown deposit of a defined compound, Cu_2Cl_2,6β-picoline, is formed, and this can either be recrystallized or it can be decomposed with sodium hydroxide and the precipitation can be repeated giving a very pure β-picoline.

Impure γ-picoline can, except through the hydrohalogenide, be purified with *o*-phenylphenol according to Bowman (20), and with benzaldehyde and $ZnCl_2$ according to Bailey *et al.* (21).

Impure 2,6-lutidine is best purified according to Riethof (22) by way of its double compound with urea or thiourea. The base can be distilled free from the urea.

The 2,4-lutidine and the 2,5-lutidine give, without forming azeotropes, the so called 2,4-fraction, which cannot be separated by distillation. The best way to sepa-

rate them is the fractionated precipitation of the hydrohalogenides according to Cislak *et al.* (23) and Engel (24).

Raw pyridine from gasworks gives a rather pure 2,3-lutidine in the fraction 160–162°C, which can be purified with urea according to Milner (25).

The best way of manufacturing 2,4,6-collidine from the fraction boiling at 170–172°C is through the hydrohalogenide as described by Engel (26). Every pyridine homologue mentioned here with substituents in α or γ, that is all except β-picoline, can be synthesized according to Rieger (27) with very good results from the bases either with lead tetraacetate in the presence of methanol or with red lead, glacial acetic acid and acetic anhydride in the presence of methanol.

The pure bases can be stabilized and finally purified by boiling with a 40 % solution of sodium hydroxide according to Waldron (28).

The methods mentioned here have been carefully investigated and the universal procedure according to Engel (18, 24, 27) has been chosen as fit at least for operating on a smaller scale. There are no difficulties with the apparatus, the procedure is very easy to handle and it gives very pure products especially after being repeated. The raw pyridine has been fractionated in a good column and the fractions obtained have been treated with dry hydrogen chloride gas in small aluminium vessels with ordinary lids. Beneath the lids we placed a simple cooler consisting of a double coil of the same material as the vessel. There are no corrosion difficulties because the hydrogen chloride gas is dry and shall never be used in excess. The reaction is exoterm and after some initial warming we usually needed outside cooling later on. The hydrochloride crystallized during the night and was then centrifugated off from the mother liquid, washed with toluene and decomposed with a 40 % solution of sodium hydroxide. Finally the base was dried and distilled.

The bases used for the present work have been purified as follows: Pyridine and α-picoline by distillation in a very good column, β-picoline through its copper chloride compound, γ-picoline by repeated precipitation of the hydrochloride, decomposition and distillation, 2,6-lutidine through its urea compound, 2,4-lutidine, 2,5-lutidine and 2,4,6-collidine in the same way as γ-picoline and 2,3-lutidine through the urea compound.

Most of the bases have been manufactured pure according to Rieger (27) and all bases have been stabilized by boiling with a 40 % solution of sodium hydroxide. They have been dried with BaO, and analysis according to Fisher has shown a water content beneath 0.1 %.

The distillation of the bases has been performed according to Skärblom *et al.* (29) and about 50 % in the middle of each fraction with a constant refractive index (20°C, sodium light) has been used.

The analysis of pyridine bases

A great deal of work has been spent in working out a reliable method of analysis. Manufacture of pure bases is possible, only if determination of the components can be carried out with sufficient accuracy. A great many methods have been described in this connection but no one of them is so simple as to be suitable from a technical viewpoint.

Gravimetric or colorimetric methods depending on usually coloured compounds between the bases and either acids or substances apt to give addition compounds with the bases are frequently met with. Sah *et al.* (30) showed that 2,4- or 3,5-dini-

trobenzoic acid and 3,5-dinitro-*o*- or *p*-toluic acid give specific compounds with pyridine bases, and Dass *et al.* (31) showed that violantine, alloxantine, violuric acid, thiovioluric acid and 1,3-diphenyl, 1,3-ditolyl or 1,3-dixylyl violuric acids or thiovioluric acids give the same. Wanag (32) and Christensen *et al.* (33) used 2-nitro-1,3-indandione, Janz *et al.* (34) picric acid, and Sakamoto (35) substituted benzenesulfonic acid chlorides.

Another method is the decomposition of the bases by UV-light reported by Freytag *et al.* (36). This method has been tried with very poor results. The same author (37) has later shown that pyridine can be degraded to glutaconic aldehyde, which gives very sensitive colour reactions with aromatic diamine hydrochlorides, but the reaction is specific for pyridine and not quantitative.

Angyal (38) uses the partial miscibility of lutidines with water for an analytical method. The cloud point is determined in a solution by means of a freezing-point apparatus; the composition of the base mixture is obtained from a standard curve and a determination of the density. The method is not very exact and it requires very dry base mixtures.

Infrared and ultraviolet spectrographic determinations have been carried out by Coulson *et al.* (39), Herington (40), Le Rosen *et al.* (41) and Ceselli (42), and they all report that the adsorption maxima are so close to each other, that multicomponent systems are very hard to analyze.

Chromatography has been used in some methods. Walker (43) eluates the hydrochlorides of the bases with *n*-butanol saturated with 2 *N* hydrochloric acid. Jerchel *et al.* (44) oxidize the bases to the corresponding acids or aldehydes with SeO_2 or $KMnO_4$ and separate them on paper from a concentrate with sec. butanol–formic acid–water (75:15:10). The acid spots are made visible with fluorescein in UV-light. The same method is more fully investigated by Kuffner *et al.* (45). Oxidation with hydrogen peroxide in acetic acid (11 %) and elution with benzene–formic acid–water (75:15:10) is also used by Jerchel *at al.* (44), and gives spots of N-oxides, which are made visible by a weak solution of acridine. Walker's method is not very accurate and the two latter suffer from the fault of not working with the bases themselves, which involves additional problems when analyzing mixtures.

Vignes *et al.* (1) make use of coloured compounds between the bases and $CuCl_2$, either on paper impregnated with a solution of $CuCl_2$ (5 g) in glycerol (25 g) and water (20 g), or in columns with Al_2O_3 (25 g) impregnated with $CuCl_2$ (2 g), water (2 g) and glycerol (6 g) and dried. Elution is effected with diethylether. Pyridine gives a blue colour, α-picoline a green, β-picoline a orange-brown, γ-picoline a dark blue, and 2,6-lutidine a violet colour, and the length of the bands is said to be proportional to the amounts of bases within 1–2 %. The paper method has been more fully investigated by Baudet (46) working with a more diluted solution for impregnation; 2,3-lutidine gives a yellow, 2,4-lutidine a faint bluish-violet and 2,5-lutidine a faint blue colour. Hisamura *et al.* (47) have modified the method with Al_2O_3 according to Vignes. After elution with light petrol the bands are dug out of the columns and decomposed with a solution of sodium hydroxide. The bases are distilled into a known quantity of hydrochloric acid and determined by titration. However, determination of β-picoline was not possible in this way.

Concerning determination of the pure bases there are only a few analytical methods mentioned in literature. Since a great deal of work has been done according to a method of Karavaev *et al.* (48), supposed to be universal, some words must be said about it. A neutral solution of the base to be determined is treated with bromothio-

cyanogen in the presence of a saturated aqueous solution of aniline. A yellow colour developes which can be determined colorimetrically. However, this method has been shown to be almost specific to pyridine, and mixtures of pyridine bases cannot be analyzed. Furthermore, a lot of preliminary work is needed in order to obtain the necessary standard curves.

The best method of determining the purity of a base is the very accurate freezing point method of Herington *et al.* (49). The best values of the physical constants of the bases have been determined by Eguchi (2) and Herington (50).

As mentioned before, the chromatographic method of Vignes *et al.* (1) has been found to be the most promising one. However, the observations of the present author did not agree at all with those reported by Vignes, and for that reason a modification of the method was worked out and is presented in this article. Since the method involves the compounds of the bases with $CuCl_2$, these have been synthesized and have been found to be, in almost every case, very well-chrystallizing compounds of which only $CuCl_2,6Py$, stable beneath $-10°C$, $CuCl_2,2Py$ and $2CuCl_2,3Py$, stable above $+58°C$, have been described earlier. A Cu compound has also been prepared from a synthetic base, 4-ethylpyridine (Organic Syntheses 27). All compounds have been analyzed by iodometric Cu-determination (the base distilled off), base determination (distillation into a known quantity of hydrochloric acid) and Cl-determination according to Volhard (base and Cu being removed). Methanol waterfree, according to Grignard, and in special cases benzene have been found to be suitable solvents.

The compounds of the pyridine bases with $CuCl_2$

I. Pyridine.—From a weak solution of $CuCl_2$ in methanol and an excess of pyridine in relation to the $CuCl_2$, dark blue parallel-epipedical rods chrystallize. The compound, which is very unstable when dry, has the composition $CuCl_2,4C_5H_5N$. It decomposes in air to a blue-green powder of $CuCl_2,2C_5H_5N$. This compound can also be prepared from the required quantities of $CuCl_2$ and pyridine in methanol, and crystallizes then as beautiful blue-green needles. The compound is stable at room temperature but decomposes on warming to 60°C, without giving any defined compound with a lower content of pyridine.

Experiments were made to prepare a compound $CuCl_2,nC_5H_5N$ ($n < 2$) in benzene, in which $CuCl_2$ is only slightly soluble, but without success. Chrystallization at higher temperatures has not given any results either.

II. α-picoline.—When α-picoline is added to a solution of $CuCl_2$ in methanol, there is formed immediately a deposit of olive-green shining scales having the composition $CuCl_2,C_6H_8N$. If the mother liquor is mixed with more α-picoline and left to crystallize, rhombohedrical dark blue-violet crystals of $CuCl_2,2C_6H_8N$ separate. This compound also crystallizes from a solution of the required amounts of $CuCl_2$ and α-picoline in methanol; it is rather stable at ordinary temperature.

$CuCl_2,C_6H_8N$ can be prepared from α-picoline in benzene with $CuCl_2$ and evaporation of the hydrocarbon.

III. β-picoline.—$CuCl_2$ with a large excess of β-picoline in methanol at first yields beautiful faintly blue needles of $CuCl_2,2C_6H_8N$ and the same compound can also be prepared from the required amounts of $CuCl_2$ and 2 moles of β-picoline in methanol. If still more β-picoline is added to the mother liquor from the first crystallization the solution becomes dark blue, and slow evaporation in air gives beautiful dark blue parallelepipedical rods of the very unstable $CuCl_2,4C_6H_8N$.

In addition to the stable $CuCl_2,2C_6H_8N$ some faint yellow-brown scales of $CuCl_2$, C_6H_8N can be obtained from benzene. The same compound deposits along the walls of the beaker at the surface of the methanol solution, probably owing to evaporation of β-picoline.

IV. γ-picoline.—With a large excess of γ-picoline a solution of $CuCl_2$ in methanol immediately becomes very dark blue and at the same time blue needles of $CuCl_2$, $2C_6H_8N$ separate. Slow crystallization of the mother liquor with more γ-picoline gives beautiful dark blue square crystals of $CuCl_2,4C_6H_8N$. Still more beautiful and well-defined crystals of the same compound separate from a weak solution of $CuCl_2$ in methanol with a large excess of 2,6-lutidine contaminated with γ-picoline. The compound is much more stable than the corresponding β-picoline derivative but still too unstable to be stored in air.

Required amounts of $CuCl_2$ and 2 moles of γ-picoline give beautiful blue needles of $CuCl_2,2C_6H_8N$ from methanol; the crystals are somewhat darker and more lustrous than those of the corresponding β-picoline derivative.

From benzene solution a compound $CuCl_2,C_6H_8N$ can be obtained. However, it does not give any form of crystals but separates in an amorphous form.

V. 2,6-lutidine.—When 2,6-lutidine is added to $CuCl_2$ in methanol beautiful faint brown scales of $CuCl_2,C_7H_{10}N$ precipitate immediately. The scales are somewhat lustrous and have rather regular corners. When the mother liquor, with some more 2,6-lutidine added in excess, is left to slow crystallization dark red-violet rhombohedra separate, having the composition $CuCl_2,2C_7H_{10}N$.

From benzene both compounds can be obtained, but the normal one is $CuCl_2$, $C_7H_{10}N$.

VI. 2,4-lutidine.—With 2,4-lutidine and an excess of $CuCl_2$ in methanol dark red-brown scales of $CuCl_2,C_7H_{10}N$ deposit. With 2,4-lutidine in excess dark blue to blue-violet small rods, partly clustered in star- or rosette-form can be obtained in slow crystallization. The composition is $CuCl_2,2C_7H_{10}N$. From benzene solution both compounds can be prepared, but the normal one is $CuCl_2,C_7H_{10}N$.

VII. 2,5-lutidine. With a deficit of 2,5-lutidine red brown scales of $CuCl_2,C_7H_{10}N$ deposit, which have a somewhat fainter colour than the corresponding 2,4-lutidine derivative and with a slight violet shade.

2,5-lutidine in excess to $CuCl_2$ in methanol gives, in slow crystallization, thin rombic plates of a blueish red-violet colour and with the composition $CuCl_2,2C_7H_{10}N$.

From benzene solutions both compounds can be obtained, but the normal one is $CuCl_2,C_7H_{10}N$.

VIII. 2,3-lutidine.—With a deficit of 2,3-lutidine dark yellow-brown scales consisting of $CuCl_2,C_7H_{10}N$ are obtained. With an exess of 2,3-lutidine $CuCl_2,2C_7H_{10}N$ crystallizes as dark violet square crystals or rhombic plates.

With benzene the same result as for, e.g., 2,4-lutidine and 2,5-lutidine.

IX. 4-ethylpyridine.—With a deficit of 4-ethylpyridine faintly brown scales of $CuCl_2,C_7H_{10}N$ deposit. With an excess of the base dark blue parallelepipedical thick rods with the composition $CuCl_2,2C_7H_{10}N$ crystallize. From benzene only scales of the first mentioned compound can be prepared.

X. 2,4,6-collidine.—With a deficit of the collidine in methanol brownish yellow-red scales of $CuCl_2,C_8H_{12}N$ deposit. With an excess of the base faint red-violet rods with the composition $CuCl_2,2C_8H_{12}N$ are obtained. Only the first mentioned compound can be prepared from benzene solution.

A common observation is that the presence of alcohols with a boiling point in the

proximity of or exceeding that of the bases makes it hard, and in the case of glycol or glycerol impossible, to crystallize the compounds. The base evaporates so fast as to give only a solution of $CuCl_2$ in the alcohol.

The compounds have no defined melting points, but decompose on heating with the formation of a brown deposit of $CuCl_2$.

The compounds are soluble in water with decomposition and therefore cannot be prepared from aqueous solution, basic salts of copper being deposited. The compounds are soluble in alcohols, dioxan, ketones, esters, aromatic hydrocarbons and aliphatic and aromatic halogen-substituted hydrocarbons. They are slightly soluble in ethers and light aliphatic hydrocarbons but insoluble in saturated aliphatic hydrocarbons with more than 5 C-atoms as also in hydroaromatic hydrocarbons.

Examination and modification of the analytical method

According to Vignes *et al.* (1) a column filling is prepared in the following way: 20 g $CuCl_2$ is dissolved in 20 g water, and 60 g glycerol is added. In a mortar 250 g Al_2O_3 is worked into the solution in small portions with the pestle. After thorough mixing the product is dried at 50°C and finally forms a faintly green light-flowing powder, absolutely dry to the touch.

The chemicals used in the present investigation were proved to be of a very good quality. The Al_2O_3 was standardized according to Brockmann and water-free $CuCl_2$ was prepared from $CuCl_2,2H_2O$ by drying at 150°C to constant weight.

The preparation of the column filling was very difficult, and it seems hardly possible to achieve an even distribution of the copper salt on the Al_2O_3 in the proposed way. Mixing in a ballmill was tried, it gave a very good distribution of the $CuCl_2$ but the flow rate of the liquid in the column was too slow for this sort of powder, and too much grinding in the mortar also impairs the result.

The powder was filled in glass tubes with an inner diameter of 3 mm and ca. 30 cm in length. 0.5 ml of the so-called β-fraction was supplied on the top. Elution with diethylether gave very short elution bands with blue and violet colour but with such a small brown band, that it could not be a measure of the content of β-picoline. The elutions always showed irregularities, and wellshaped, even bands necessary for a quantitative determination were never formed.

A great number of elution liquids was tested; the experiments showed that only a special rather high-boiling petrol or hydro-aromatics gave indeed not ideal but anyway good elutions, and as expected without any brown bands at all.

The preparation of the column fillings now was altered in such a way that $CuCl_2$ was brought into solution with a great many different alcohols, with or without the addition of water. Dry filling as well as filling with a suspension of Al_2O_3 in the $CuCl_2$ solutions and washing with the elution liquid were tested. It was found, that a solution of $CuCl_2$ in waterfree methanol gave the most promising results, and high boiling petrol was shown to be the best elution liquid.

In all cases the elutions showed a tendency to stop at band-lengths proportional to the test quantities (see Table 1) but with all elution liquids, other than the best one mentioned, the compounds later on began to dissolve or thin off and brown colours appeared partly in the boundary area between the blue and the violet and partly beneath the violet band. The same tendency appeared when the powder was prepared with higher alkohols, especially with glycol and glycerol, in the Cu solution, even if

Table 1

Test β-fract. ml	Total elution length cm	Violet colour, 2,6-lutidine cm	Blue colour I, γ-picoline cm	Blue colour II, β-picoline cm
0.2	12.8	4.8	3.2	4.8
0.4	25.0	9.5	6.1	9.4
0.6	37.4	14.1	9.2	14.1

petrol was used as the elution liquid. It seems that the eluents not only dissolve the compounds but also at least partly prevent the forming of the same.

The experiments of Table 1 were carried out with glycerol in the powder and with petrol elution. The thinning out is most marked in the 0.2 ml test and can best be seen in the band-lengths of the 2,6-lutidine. However, it can be observed that the band-lengths are roughly proportional to the quantities of bases.

In further experiments the amount of $CuCl_2$ in the filling was varied, and the tests gave the interesting result, that the length of the bands varied with the Cu content in the way of being shorter at larger Cu concentrations. In various experiments, e.g. with columns divided in sections by pieces of cotton-wool, it was found, as was expected, that the method is not a real chromatography, but can be characterized as a saturation of the $CuCl_2$ with bases through the formation of defined Cu compounds of different colours, the compositions of which are dependent on the concentration of the base and of the copper salt.

A comparison between these observations and the properties of the above-mentioned rather stable Cu compounds with the bases, explains these results. The quantities of the different bases cannot be calculated directly from the band-lengths but their molecular weights must be taken in consideration. The β-picoline does not give any brown or yellow-brown colours; the bands only depend on the formation of Cu_2Cl_2, 6β-picoline at large Cu and base concentrations and of $CuCl_2$,β-picoline at low ones. The results with the different elution liquids can be explained in the same way, they influence the formation of the compounds in different ways.

If the sample was put into the column directly on top of the powder a formation of $CuCl_2$, 4 γ-picoline could be seen, but if direct contact between undiluted sample and filling was prevented with a piece of cotton-wool or if the samples were diluted with petrol before adding, very even elutions resulted, the bands showing the colours of the stable Cu compounds. In order to get even elutions of the right length it proved necessary to use a certain optimal concentration of $CuCl_2$ in the filling. The results concerning the compounds of the pyridine bases with $CuCl_2$ also show why the method of Hisamura *et al.* (47) cannot yield good values for β-picoline, because they tried to distill their β-picoline from brown bands.

Elution in vacuum has been tried with bad results; this is understandable since the analysis is a saturation method, so that the vapour pressure of the bases influences the bands; they become too large and with brown spots in them.

In order to improve the method, $CuCl_2$ was dissolved in methanol and Al_2O_3 was added in the calculated quantity. After the powder had been dried the Cu content was determined (iodometrically) and it was found that a very even impregnation had been obtained. The powder was weighed into the tubes and these were knocked against

Table 2

Mole $CuCl_2$/cm tube length	Mole $CuCl_2$/g powder	Base found/ base used	Violet colour, 2,6-lutidine		β-picoline cm	γ picoline cm
			cm	cm $\times \frac{107}{93}$		
0.0129	5.46×10^{-4}	0.91	6.2	7.1	6.7	4.8
0.0114	4.19×10^{-4}	1.00	7.5	8.6	8.4	6.1
0.0088	3.25×10^{-4}	1.07	10.6	12.2	11.4	8.1
0.0063	2.36×10^{-4}	1.14	15.1	17.4	17.5	12.7

a piece of wood until no decrease of the column length could be seen (a plug of cotton-wool in the bottom end of the tube). Measurements on the same tube showed a constant ratio of column length and weight of the powder. No sinking of the upper area nor any interruptions of the column could be seen when the elution liquid was passed through the tubes.

Using columns carefully prepared by weighing it was shown that for the elution an optimum of the Cu concentration in the powder could be deduced. (Table 2 and Fig. 1.) This optimum was at about 4×10^{-4} mole $CuCl_2$/g powder. If the bandlengths were calculated in respect to known Cu compounds with the molecular weights of the bases taken into consideration a very good agreement was found between the weight of bases calculated and the amounts used.

The composition of the used factory fraction in respect to β-picoline, γ-picoline and 2,6-lutidine, calculated from the analytical results, was 37.2 % 2,6-lutidine, 36.4 % β-picoline and 26.4 % γ-picoline, and these values are in very good agreement with the actual yields of the bases, obtained from this fraction. The fraction has also been analyzed by infrared spectrography, but the results were less satisfactory.

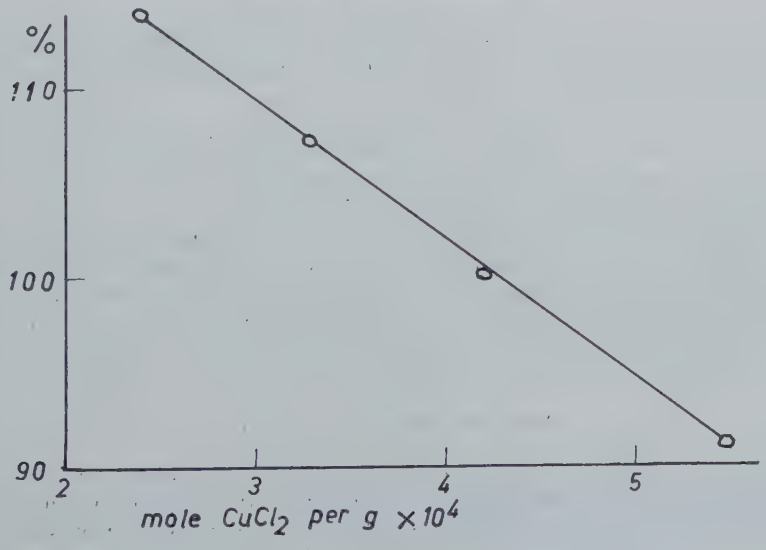

Fig. 1.

For 2,6-lutidine and bases with still higher boiling points the colours of the Cu compounds are so much alike, that it is very hard to see any difference between them, except for the 4-ethylpyridine compound, which has a blue colour just as the γ-picoline compound. There is reason to assume that also the 2-ethylpyridine and 3-ethylpyridine compounds have the same colours as the α-picoline and β-picoline derivatives. With some training it is very well possible to see the limits between the bands of 2,6-lutidine, 2,4-lutidine and 2,5-lutidine, 2,3-lutidine and 2,4,6-collidine, but it must be recommended to obtain as good separation of the bases as possible by distillation before the analysis. These distillations also are of great value in preparing the pure bases.

The difference in colours of the β-picoline and γ-picoline compounds can easily be seen also by an untrained person.

All pure bases in question have been analyzed with the method outlined here, and the results were very good. Analysis of weighed test compositions have also shown very good agreement within 1–2 % of the values.

The different bases when treated under proper conditions show the following colours:

Pyridine	Blue-green	2,4-lutidine	Dark blue violet
α-picoline	Dark blue-violet	2,5-lutidine	Faint bluish red-violet
β-picoline	Faint blue-green	2,3-lutidine	Faint greyish red-violet
γ-picoline	Faint clear blue	2,4,6-collidine	Faint red-violet
2,6-lutidine	Dark red-violet	4-ethylpyridine	Faint clear blue

Since the compounds of the higher bases investigated all have violet colours, there is reason to assume that for the still higher bases the colour differances will be more and more blotted out.

Chromatography on paper

According to Vignes *et al.* (1) and Baudet (46) the method mentioned can be used in chromatography on paper strips, prepared by impregnation with an appropriate solution and dried.

A great many tests have been carried out, but with very bad results and a quantitative method seems impossible to realize. When the test quantity is placed on the paper strip, the $CuCl_2$ near the drop will be saturated with base, giving the Cu compound with the highest possible base content, and as we have seen these are unstable and give off vapour of the bases, which as a matter of fact makes such a method impossible especially for low boiling bases. With more high boiling bases better results were obtained, obviously because the highest possible Cu compounds here are much more stable and contain only 2 mole of base. These bases also have a much lower vapour pressure, but nevertheless the results were unsatisfactory.

The new analytical method

20 g water-free $CuCl_2$ is dissolved in 125 g water-free methanol, and 250 g Al_2O_3 standardized according to Brockmann is stirred into the solution in small portions to a very homogeneous mixture. The product is dried for 8 hours at 50–60°C (during the first two hours the powder is stirred twice an hour). The Al_2O_3 now impregnated

with $CuCl_2$ is a yellow-brown powder with the same grain-size distribution and free flowing properties as the original material; the Cu content is determined iodometrically. The powder is now ready for use and can be stored for any time in closed containers.

Glass tubes with an inner diameter of about 3 mm and with a normal wall thickness are used as columns. At the upper end of the tube there is a cylindrical container holding about 50 ml, and the tube is about 30 cm in length. (The dimensions may be altered, if required.) The tubes must be absolutely straight and the inner diameter must not vary.

A plug of cotton-wool is put into the lower end of the tube and a suitable quantity of the powder is filled into it. The tubes are knocked against a firm piece of wood until the upper area of the column does not sink any more. Another plug of cotton-wool is pressed against the top of the column and the tube is knocked a few more times until further pressing of the plug does not move it.

The sample, 0.2–1.0 ml base-fraction, well distilled, is weighed into a small flask with a ground glass stopper, diluted with petrol and transferred to the holder of the tube with the aid of petrol (total volume 30 ml), or it can be measured by volume into a quantity of petrol and be transferred to the holder in the same way. In the first case the test quantity can be directly controlled and through it the quantities of the absorbed bases, if one knows the percentage of Cu in g or mole/cm. In the latter case, which is easiest in routine work, the composition of the samples can be taken from the band lengths multiplied by the molecular weight ratios.

If the sample contains γ-picoline in a quantity larger than about 20 % a second elution must be made with about 10 ml petrol. This is to be sure, that all of the posssibly formed compound of $CuCl_2$ with 4 γ-picoline, will be decomposed. In special cases the necessary quantity of petrol must be determined in separate tests and, in order to get very good results, a curve of the type shown in Fig. 1 has to be prepared.

Whether the samples are eluted in the correct way, can easily be seen by a comparison of the band colours with those mentioned earlier.

The column fillings are rather difficult to clean out from used tubes, as they stick firmly to the walls. A good method is to use a brass tube sharpened at one end and with a diameter slightly smaller than the inner diameter of the glass tube. The brass tube is drilled into the column and at the same time water is lead through it; the tubes are cleaned in a few seconds in this way.

The preparation of pure bases

Tubes with larger diameter ($\sim$ 20 mm) are filled with the powder, and base mixture in petrol are fed into the columns. By digging out the bands, decomposing the compounds with sodium hydroxide solution and following steam distillation very pure bases can be produced. A technical manufacture of the bases cannot be based on this method but in the laboratory a base fraction can easily be separated in its components. The elution petrol can very well be filled into the holder of the tube with a so called chicken automaton.

Summary

A modification of a method of Vignes *et al.* (1) and Baudet (46) for separation and determination of mixtures of pyridine derivatives has been worked out, which is found to be rather simple and to give accurate values to within 1–2 %.

The saturation of a substance (e.g. $CuCl_2$) on a suitable carrier (e.g. Al_2O_3) with a sample consisting of compounds forming addition compounds with different affinities, can be used to obtain a complete separation of the components. Methods of this type seem never before to have been reported and may prove valuable in other fields of chemistry.

A detailed description of the method is given both for analytical purposes and for laboratory preparation of pyridine bases.

Several new addition compounds of $CuCl_2$ and pyridine bases have been synthesized and their compositions have been proved by analysis. They are, with few exceptions, beautifully coloured and most of them crystallize well, but they are unstable and have no defined melting points.

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REFERENCES

1. R. Vignes and P. Chervet, Compt. Rend. *232*, 1419 (1951).
2. T. Eugchi, Bull. Chem. Soc. Japan *2*, 176 (1927); *3*, 227 (1928).
3. R. Benzie, J. Milne, and H. Nisbet, Oil, Shale and Cannel Coal, 2nd conf. *II*, 770 (1951).
4. Y. Fushizaki, Technol. Repts. Osaka Univ. *I*, 309 (1951).
5. J. Dodonow and E. Soschestwenskaja, Ber. *62*, 1348 (1929).
6. J. Hackmann and J. Wibaut, Rec. Trav. Chim. *62*, 229 (1943).
7. A. Bratton and J. Bailey, J. Am. Chem. Soc. *59*, 175 (1937).
8. R. Mahan and J. Bailey, Ibid. *59*, 2449 (1937).
9. B. Densham, J. Langston, and J. Simpson, J. Chem. Soc. *1952*, 5044.
10. H. Winkler, Das Steinkohlenteer. Glückauf, Essen (1951).
11. A. Lidstone, J. Chem. Soc. *1940*, 241.
12. K. Engel, U.S. Patent 2,408,975 (1940).
13. K. Slagle and R. Bowman, U.S. Patent 2,459,191 (1947).
14. L. Cracas, U.S. Patent 2,516,370 (1946).
15. F. Cislak and O. Cunningham, U. S. Patent 2,374,418 (1941).
16. G. Riethof, U.S. Patent 2,383,016 (1944); U.S. Patent 2,412,649–51 (1945).
17. E. Coulson and J. Jones, Brit. Patent 585, 108 (1947).
18. K. Engel, U.S. Patent 2,420,529 (1942).
19. U. Zavody, N. Podnik, and L. Rusek, Austrian Patent 172,652 (1952).
20. R. Bowman, U.S. Patent 2,459, 416 (1947).
21. R. Barley and S. McElvain, J. Am. Chem. Soc. *52*, 1633 (1930).
22. G. Riethof, U.S. Patent 2,295,606 (1941); U.S. Patent 2,325,880 (1942); U.S. Patent 2,376,008 (1943).
23. F. Cislak and M. Otto, U.S. Patent 2,456,377 (1943).
24. K. Engel, U.S. Patent 2,510,876 (1946).
25. D. Milner, Brit. Patent 584,148 (1947).
26. K. Engel, U.S. Patent 2,426,442 (1942).
27. W. Rieger, U.S. Patent 2,502,174 (1947).
28. J. Waldron, U.S. Patent 2,454,019 (1943).
29. K.-I. Skärblom and Å. Linder, Teknisk Tidskrift, Chemistry *67*, 12–15 (1937), Stockholm, Sweden.
30. P. Sah and C. Tien, J. Chinese Chem. Soc. *4*, 490 (1936).
P. Sah and K. Yuin, Ibid. *5*, 129 (1937).

31. I. Dass and S. Dutt, Proc. Indian Acad. Sci. *8A*, 145 (1938).
32. G. Wanag, A. Lode, and A. Dombrowski, Ber. *70B*, 547 (1937); ibid. *75B*, 82 (1942).
33. B. Christiansson *et al.*, Anal. Chem. *21*, 1573 (1949).
34. G. Janz and R. Salomon, Ibid. *25*, 454 (1953).
35. S. Sakamoto, J. Pharm. Soc. Japan *71*, 173 (1951).
36. H. Freytag and W. Neudert, J. Prakt. Chem. *135*, 15 (1932).
37. H. Freytag, Z. Anal. Chem. *142*, 12 (1954).
38. S. Angyal, Australian Chem. Inst. J. and Proc. *14*, 12–16 (1947).
39. E. Coulson *et al.*, J. Applied Chem. *2*, 71 (1952).
40. E. Herington, Discussions Faraday Soc. *9*, 26 (1950).
41. H. Le Rosen and J. Wiley, Anal. Chem. *21*, 1175 (1953).
42. C. Ceselli, Chimica e Industria Milan *36*, 595 (1954).
43. W. Walker, Australian J. Science *13*, 26 (1950).
44. D. Jerchel and W. Jacobs, Angew. Chem. *65*, 342 (1953); *66*, II, 298 (1954).
45. F. Kuffner and N. Faderl, Monatsh. *86*, 995–1003 (1955).
46. J. Baudet, Compt. Rend. *234*, 2454 (1952).
47. M. Hisamura *et al.*, Coal Tar Japan *6*, 45 (1954).
48. N. Karavaev and I. Vener, Izvest. Akad. Nauk. SSSR, Otdel. Tekh. Nauk. *1947*, 913 Fuel Abstracts, *3*, 105 (1948).
49. E. Herington and R. Handley, J. Chem. Soc. *1950*, 499.
50. E. Herington and J. Martin, Trans. Faraday Soc. *49*, 154 (1953).

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